Evaluation of Potential Agricultural Applications of the Microalga *Scenedesmus dimorphus*

by

Jesus Garcia-Gonzalez

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved November 2014 by the
Graduate Supervisory Committee:

Milton Sommerfeld, Chair
Kelly Steele
Mark Henderson

ARIZONA STATE UNIVERSITY

December 2014

ABSTRACT

Microalgae represent a potential sustainable alternative for the enhancement and protection of agricultural crops. The dry biomass and cellular extracts of *Scenedesmus dimorphus* were applied as a biofertilizer, a foliar spray, and a seed primer to evaluate seed germination, plant growth, and crop yield of Roma tomato plants. The dry biomass was applied as a biofertilizer at 50 g and 100 g per plant, to evaluate its effects on plant development and crop yield. Biofertilizer treatments enhanced plant growth and led to greater crop (fruit) production. Timing of biofertilizer application proved to be of importance – earlier 50 g biofertilizer application resulted in greater plant growth. *Scenedesmus dimorphus* culture, growth medium, and different concentrations (1%, 5%, 10%, 25%, 50%, 75%, 100%) of aqueous cell extracts were used as seed primers to determine effects on germination. Seeds treated with *Scenedesmus dimorphus* culture and with extract concentrations higher than 50 % (0.75 g ml^{-1}) triggered faster germination – 2 days earlier than the control group. Extract foliar sprays of 50 ml and 100 ml, were obtained and applied to tomato plants at various extract concentrations (10%, 25%, 50%, 75% and 100%). Plant height, flower development and number of branches were significantly enhanced with 50 % (7.5 g ml^{-1}) extracts. Higher concentration sprays led to a decrease in growth. The extracts were further screened to assess potential antimicrobial activity against the bacterium *Escherichia coli* ATCC 25922, the fungi *Candida albicans* ATCC 90028 and *Aspergillus brasiliensis* ATCC 16404. No antimicrobial activity was observed from the microalga extracts on the selected microorganisms.

DEDICATION

Dedicado a mis padres Santiago y Esperanza Garcia por todo su apoyo, cariño y comprensión. Por sus invaluables consejos y su valentía de sacrificar todo para darnos a mis hermanos y a mi una vida privilegiada, la cual ustedes no tuvieron.

A mis hermanos Chago, Nora, Rica, Lupe, Tolo, Pera, Tetin y Juan por todo su apoyo y cariño.

ACKNOWLEDGMENTS

I am grateful to my mentors, colleagues, family, and friends, without whom this thesis would not have been possible. To my advisor, Milton Sommerfeld, for being so helpful and supportive throughout the past two years – for giving me full control over my research, and guiding me throughout the entire process and to AzCATI and John McGowen for providing the algae biomass used for the various applications. To my committee members – Mark Henderson and Kelly Steele, for your invaluable support, advice, and feedback.

I appreciate all that I have learned from my incredibly talented office-mates and colleagues: Shahrzad Badvipour, Emil Puruhito, Joshua Wray, Everett Eustance, and Martha Kent for the laughter, distractions, and feedback.

To my dearest friends and closest confidants Karla Alonzo, Dulce Medina, Shakira McCall, and Diana Juarez, for your immeasurable support, the everlasting memories, and endless conversations. Thank you for making this journey a wonderful ride.

I am grateful to the More Graduate Education at Mountain States Alliance (MGE@MSA) program and to the Graduate and Professional Student Association (GPSA) for partially funding my research.

TABLE OF CONTENTS

Page

CHAPTER Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER 1

INTRODUCTION

In the coming decades, a crucial challenge for humankind will be meeting future food demands without causing further environmental degradation. Agricultural systems are already major forces of global environmental pollution, accounting for 1/3 of all greenhouse gas emissions. The expanding global population, mostly in developing countries with emerging economies, and their anticipated adoption of westernized diets – heavily comprised of dairy and meat products represent added pressure to Earth's resources. Society faces a challenge to not only increase agricultural production amidst global climate change which threatens to diminish harvests in many areas of the world, but to develop a more robust food system that is sustainable, stable, and less dependent on fossil fuels.

Conventional agriculture and its perpetuance of unsustainable crop production practices has resulted in devastating environmental problems such as, soil infertility, eutrophication, biodiversity loss, ocean acidification, ocean dead zones and the decline in honey bee populations (Cresswell & Thompson, 2012). As a result of adaptation and resistance developed by pests to agrochemicals, higher dosages and newer, stronger, more toxic chemicals will be required to enhance crop production, given current production practices. Agrochemicals have undoubtedly benefited humanity by enhancing agricultural production and controlling pests and pathogens. However, aside from being a major factor in the degradation of ecosystem services, and global climate change, various studies have demonstrated a relationship between pesticide exposure and human chronic diseases such as

cancers, diabetes, reproductive health issues, and neurodegenerative disorders like Parkinson's disease and Alzheimer's disease (Androutsopoulos, Hernandez, Liesivuori, & Tsatsakis, 2013; Bouchard, Bellinger, Wright, & Weisskopf, 2010; Mascarelli, 2013; Mostafalou & Abdollahi, 2013; Parrón, Requena, Hernández, & Alarcón, 2011).

With the most productive agricultural areas already under cultivation, crop enhancement and protection products along with agroecological production systems will become even more critical in order to meet the expected food demands without causing further environmental degradation. Biofertilizers are considered an environmental friendly, cost effective, sustainable alternative to synthetic fertilizers. Biofertilizers are natural fertilizers comprised of microorganisms such as bacteria, algae, and/or fungi that improve soil chemical and biological properties, stimulate plant growth, and restore soil fertility (Abdel-Raouf, Al-Homaidan, & Ibraheem, 2012; Metting, 1990). A majority of the biofertilizer research has been focused on seaweeds (Chojnacka, Saeid, Witkowska, & Tuhy, 2012; Khan et al., 2009; Verkleij, 1992) and on plant growth-promoting microorganisms such as *Rhizobium*, *Mycorrhizae,* and cyanobacteria – mainly on rice fields, due to their abundance in soil habitats.

A number of microalgae species have been investigated for their ability to produce bioactive compounds (Borowitzka, 1995; Burkiewicz, 1987; Fogg, 1983; Foley, Beach, & Zimmerman, 2011; Pulz & Gross, 2004). Several studies have established an association between greater nutrient uptake, higher biomass accumulation and greater crop yields to the incorporation of microalgae

biofertilizers (Faheed & ABD-EL Fattah, 2008; Shaaban, 2001). Similar studies have also demonstrated a significant correlation between soaking seeds with algae extracts to generate faster seed germination and greater plant growth (Shukla & Gupta, 1967).

Microalgae represent a potential solution to mitigate global climate change because they: (i) can be cultivated in wastewater and agricultural run-off, recovering excess nutrients and reclaiming water for further use; (ii) can sequester carbon dioxide and nitrous oxides from industrial sources; (iii) and are capable of producing high lipid compounds that can be utilized for food, animal feed, and fuel, further decreasing our impact on global climate change and enabling us to transition into a more sustainable future. However, microalgae production must overcome several barriers in order for it to become economically viable (Brennan & Owende, 2010; Mata, Martins, & Caetano, 2010; Pragya, Pandey, & Sahoo, 2013; Wijffels & Barbosa, 2010). One way to make microalgae production more economically feasible given current technologies, is to find potential applications for microalgae by-products that enable producers to offset production costs.

The objectives of this study were to evaluate the potential agricultural applications of the robust and common microalga *Scenedesmus dimorphus*. This alga was selected because it has been successfully cultivated at the Arizona Center for Algae Technology and Innovation (AzCATI) for high biomass production for use as a potential biofuel and bioproducts. The microalga biomass was utilized as a solid biofertilizer, applied to Roma tomatoes (*Solanum lycopersicum* var. Roma), to determine if the biofertilizer treatments would result in greater biomass

accumulation, and higher crop yields. A second biofertilizer experiment was conducted to assess whether the timing of application would have any effects on plant growth. The *Scenedesmus dimorphus* aqueous cell extracts were also utilized as seed primers and foliar sprays. Tomato seeds were soaked in growth medium, *Scenedesmus* culture, and different concentrations of cell extracts to determine their effects on seed germination and seedling growth. Two foliar sprays in varying concentrations were applied to tomato plants and several growth parameters were observed. Additionally, the cellular extracts were screened for potential bioactivity against *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 90028, and *Aspergillus brasiliensis* ATCC 16404, to determine their ability to inhibit microbial growth.

CHAPTER 2

EFFECTS OF *SCENEDESMUS DIMORPHUS* AS A BIOFERTILIZER ON GROWTH AND YIELD OF ROMA TOMATO PLANTS

Abstract

Experiments were conducted on tomato plants to study the effects of dry *Scenedesmus dimorphus* biomass as a biofertilizer. Pre-treated (with liquid nitrogen) and untreated 50 g and 100 g biomass treatments were applied to tomato plants to assess whether biomass pre-treatment would result in enhanced growth and production in tomato plants. Pre-treated 50 g and 100 g biomass treatments did enhance crop production, but failed to significantly increase other growth parameters, demonstrating no clear correlation between biomass pre-treatment with liquid nitrogen/smashing to greater plant development. A subsequent biofertilizer experiment was performed to investigate whether the timing of the biofertilizer application into the potting soil would have any effect on the effectiveness of the biofertilizer. Treatments (50 g and 100 g) applied 22 days prior to tomato seedling transplant enhanced plant development, demonstrated by producing greater numbers of branches and flowers, and higher total fresh weight, compared to the control group and the 50 g and 100 g biofertilizer treatments applied at time of seedling transplant.

2.1. Introduction

The overuse of synthetic agrochemicals has resulted in massive ecological degradation throughout the world. From production, distribution, to field

application – agrochemicals consume vast amounts of water and fuel resources making industrial agricultural production highly unsustainable. Biofertilizers present a potential alternative to synthetic agrochemicals for they not only enhance agricultural production but also diminish environmental pollution, if properly managed. Biofertilizers are products that contain living microorganisms or natural compounds derived from organisms such as plants, fungi, bacteria and algae. Microalgae contain high levels of micronutrients and macronutrients essential for plant growth. Crops treated with microalgae have been reported to exhibit enhanced nutrient uptake, plant growth and greater crop yields (Abd El Moniem & Abd-Allah, 2008; Abdel-Raouf et al., 2012; Faheed & ABD-EL Fattah, 2008; Shaaban, El-Saady & El-Sayed, 2010; Verkleij, 1992).

Although, investigations evaluating the potential agricultural applications of microalgae are limited, there is some evidence that microalgae are able to increase crop plant biomass accumulation and lead to greater crop yields. Previous studies utilizing wet and dry microalga *Chlorella vulgaris* biomass as a biofertilizer conducted under greenhouse and field conditions demonstrated greater nutrient uptake, higher root and shoot development and greater chlorophyll concentration on treated maize plants (Faheed & ABD-EL Fattah, 2008; Shaaban, 2001). The objectives of this work were to investigate the effects of dry biomass of the microalga *Scenedesmus dimorphus* as a biofertilizer on the growth and development of Roma tomatoes (*Solanum lycopersicum* var. Roma) under greenhouse conditions.

2.2. Materials and Methods

Cultivation and harvesting

The microalga *Scenedesmus dimorphus* was cultivated outdoors in seven 4'x 48' production row panel photobioreactors (Fig. 2.1) using standard BG-11 algae culture medium (Stanier, Kunisawa, Mandel, & Cohen-Bazire, 1971), bubbled with air mixed with 1% carbon dioxide, at the Arizona State University, Arizona Center for Algae Technology and Innovation (AzCATI). Samples were taken to analyze the biomass biochemical composition. The biomass was stressed for high lipid production and was harvested by centrifugation at day 14. The biomass was then frozen until further use. The frozen biomass was thawed at 4^{o}C for 24 hours. Once thawed, the biomass was spread onto ten metal trays to a thickness of 1.5 cm and placed inside a Freeze-dryer (Millrock Max Series) at -40^{o}C and allowed to dry for approximately 48 hours. The dried biomass was then collected and stored in a cold room at 4^{o}C until further use.

Fig. 2.1 – Early Growth Phase of *Scenedesmus dimorphus* Cultivation in 4' x 48' Panel Photobioreactors

Chemical composition

Biomass samples were sent to the Arizona State University Goldwater Analytical Laboratory to analyze the biomass elemental composition. Nitrogen was analyzed through combustion analysis using a PE 2400 CHN analyzer.

Experimental design

Two biofertilizer experiments were conducted under greenhouse conditions from June to October 2014. In the first experiment, Roma tomato (*Solanum lycopersicum* var. Roma) seeds were grown using standard potting soil (vermiculite: peat moss). The seedlings were then transplanted after 15 days (once true leaves

were developed) to 28 cm pots (one seedling per pot), which contained either organic (Gro-Well) or synthetic (Miracle-Gro) potting soil, that had been mixed with respective biofertilizer treatments: 50 g and 100 g of pre-treated and untreated dry *Scenedesmus dimorphus* biomass. The pre-treated biomass was treated by freezing-and-smashing three times with liquid nitrogen on a ceramic mortar with 10 minute thawing periods in between. The synthetic potting soil received only pre-treated biomass, under the presumption that nutrients in the pre-treated biomass would be more readily available for plant uptake. The organic potting soil received both pre-treated and untreated biomass treatments to assess if there were in fact differences between pre-treated and untreated biomass biofertilizer applications. Each treatment had three replicates setup in a completely randomized block design. Plants were hand watered as needed, and grown until maturity. Plant height (cm), number of flowers, and crop yield – fresh tomato weight (g), were recorded.

Treatments:

1) Control (no biofertilizer, organic potting mix)

2) Control (no biofertilizer, synthetic potting mix)

3) T_1: 50 g of biofertilizer/organic potting mix – untreated

4) T_2: 100 g of biofertilizer/organic potting mix – untreated

5) T_3: 50 g of biofertilizer/organic potting mix – pre-treated

6) T_4: 100 g of biofertilizer/organic potting mix – pre-treated

7) T_5: 50 g of biofertilizer/synthetic potting mix – pre-treated

8) T_6: 100 g biofertilizer/synthetic potting mix – pre-treated

A second biofertilizer experiment was conducted to evaluate whether earlier application of the biofertilizer (weeks prior to transplant) would have an effect on plant growth. Two biomass treatments at 50 g and 100 g were applied 22 days prior to seedling transplant, into 28 cm pots containing standard potting soil (peat moss: vermiculite: perlite). The other 50 g and 100 g biofertilizer treatments were applied at the time of transplant. Each treatment had three replicates and was setup in a completely randomized block design. Plants were grown for a total of 55 days and were hand watered as needed. Plant height (cm), number of flowers, number of branches, and early fruit development were recorded for all treatments. One sample per treatment was then chosen at random to measure total fresh plant weight (g).

Treatments:

1) Control (no biofertilizer)

2) T_1: 50 g of biofertilizer applied 22 days prior to transplant

3) T_2: 100 g of biofertilizer applied 22 days prior to transplant

4) T_3: 50 g of biofertilizer applied at time of transplant

5) T_4: 100 g of biofertilizer applied at time of transplant

Statistical analysis

Statistical analyses were conducted using StatPlus®:mac LE programming (AnalystSoft, Inc.). All experiments were analyzed using a one-way analysis of variance (ANOVA), to test difference among the means: the level of significance was set at $P< 0.05$.

2.3. Results and Discussion

Chemical composition

The biochemical composition of the biomass contained 40% lipids, 30% carbohydrates and 23% protein. CHN analysis showed that the biomass contains 2.53 % of nitrogen per gram, meaning that per 50 g and 100 g biofertilizer applications there was about 1.25 g and 2.5 g of nitrogen, respectively.

Biofertilizer trials

In the initial biofertilizer experiment evaluating the pre-treated and untreated biomass as a biofertilizer on synthetic and organic potting soil, the results demonstrated a significant correlation between biofertilizer treatments and greater plant growth, compared to the control groups. For the 50 g and 100 g biofertilizer treatments in synthetic potting soil (Fig. 2.2), the 50 g treatment led to a slightly greater number of flowers (Fig. 2.3a), while the 100 g biofertilizer enhanced plant height (Fig. 2.3b), and led to greater crop yields, producing approximately 350 grams more of fresh tomato weight than the 50 g treatment (Fig. 2.3c).

Fig. 2.2 – Biofertilizer Added to Synthetic Potting Soil: 100 g and 50 g, Compared to Control (From Left to Right)

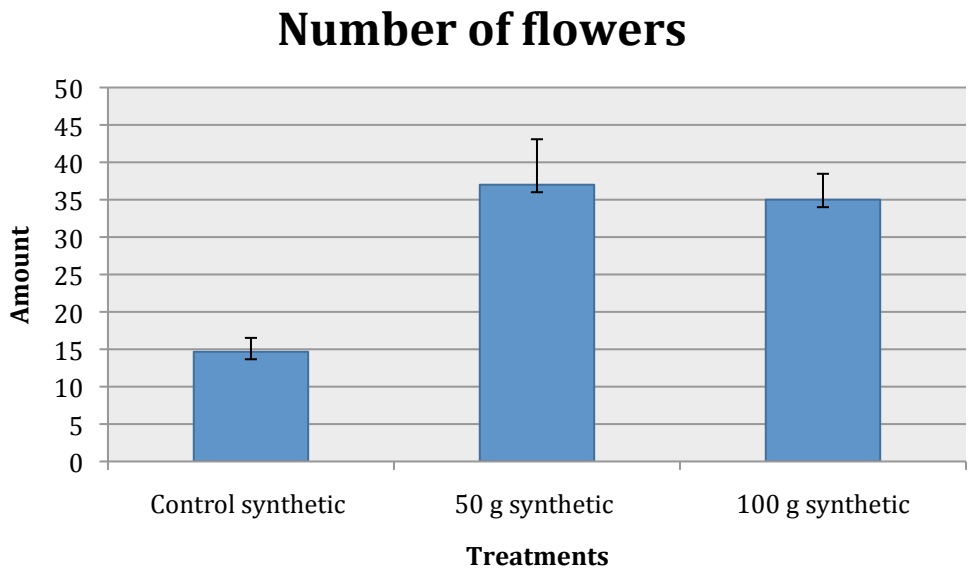

Fig. 2.3a – Biofertilizer Mixed in Synthetic Potting Soil and its Effects on Flower Development (*P*=0.0167)

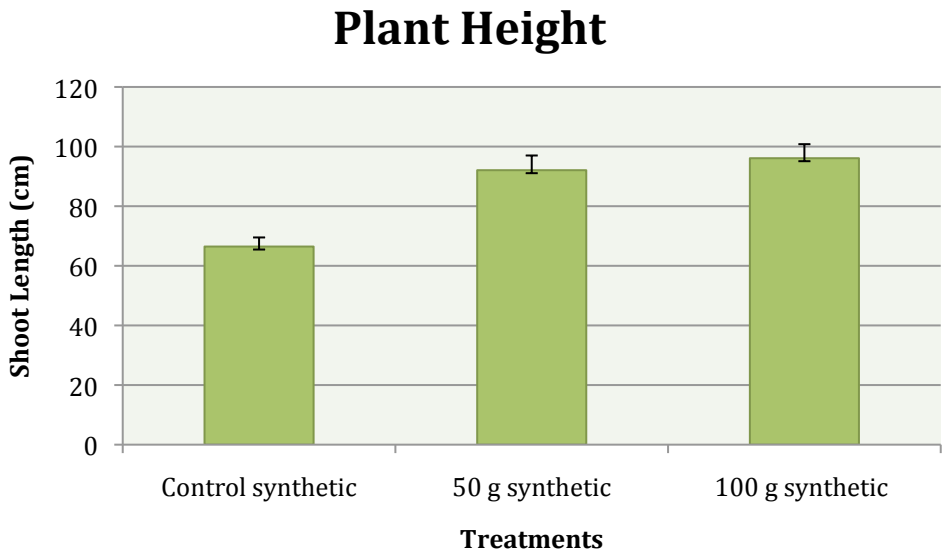

Fig. 2.3b – Biofertilizer Mixed in Synthetic Potting Soil and its Effects on Plant Height (*P*=0.0056)

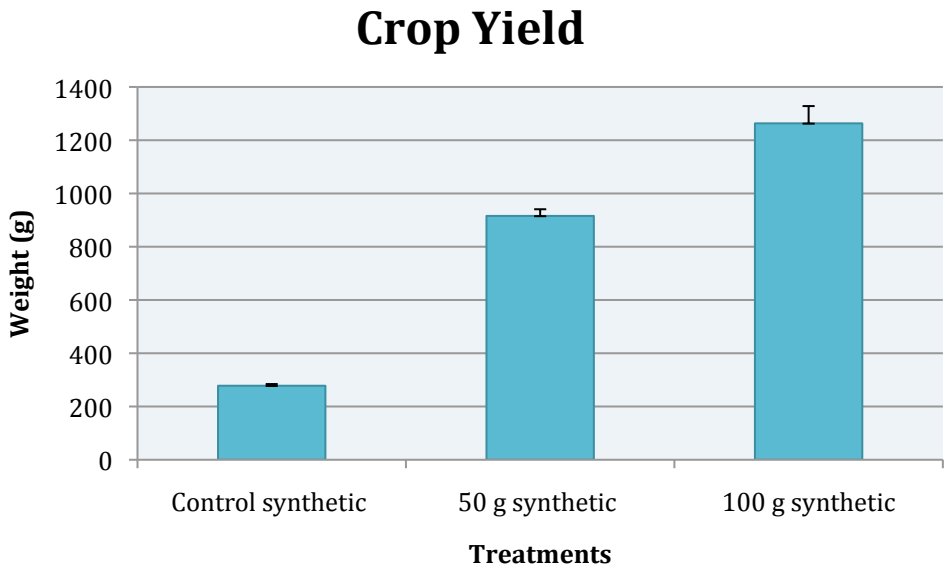

Fig. 2.3c – Biofertilizer Mixed in Synthetic Potting Soil and its Effects on Crop Yield (Tomato Weight) (*P*=0.0033)

Fig. 2.4 – Biofertilizer Added to Organic Potting Soil: 100 g Untreated, 100 g Pre-treated, 50 g Untreated, 50 g Pre-treated, Control (From Left to Right)

On the organic potting soil (Fig. 2.4) both 50 g and 100 g pre-treated and untreated biofertilizer treatments led to greater flower development (Fig. 2.5a), plant height (cm) (Fig. 2.5b), and higher crop yields, compared to the control group. The 100 g untreated biofertilizer application led to greater development of flowers, compared to other treatments. Although, minimal variation was observed in plant height between pre-treated and untreated biofertilizer applications, the untreated biofertilizer 50 g and 100g treatments had a slightly greater plant height (cm). Higher crop yields (fresh tomato weights) were obtained from the 50 g and 100 g pre-treated biofertilizer applications, more than tripling their untreated biofertilizer counterparts (Fig. 2.5c). Although, higher crop yields were obtained with the pre-

treated biofertilizer biomass treatments. When accounting for all growth parameters, there is no clear trend that shows whether the biomass pre-treated with liquid nitrogen and smashing had an effect on plant growth.

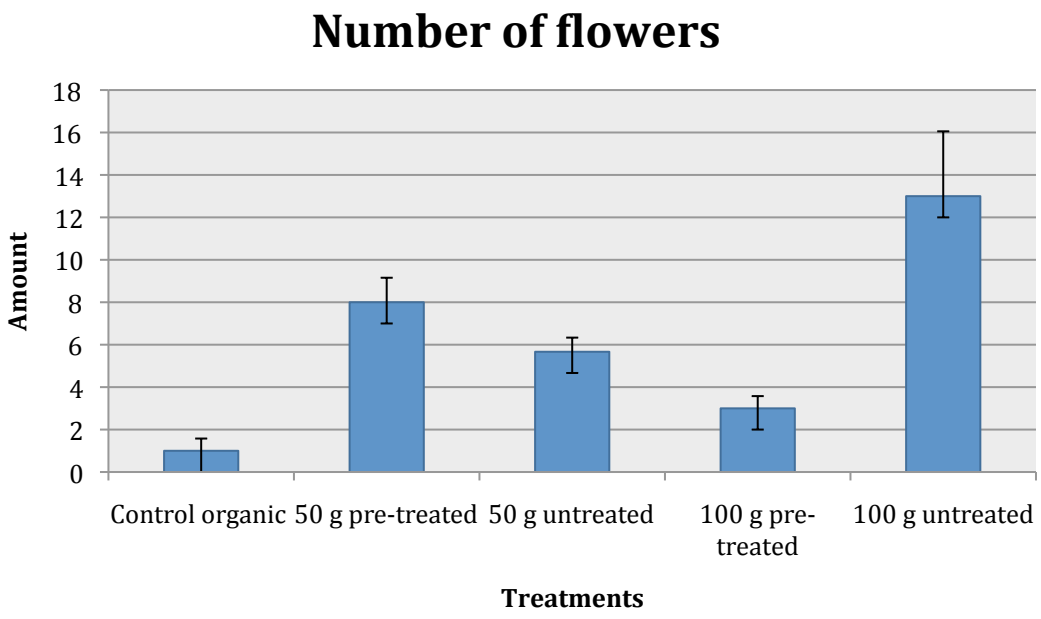

Fig. 2.5a – Pre-treated and Untreated Biofertilizer Application in Organic Potting Soil and its Effects on Flower Development (*P*=0.0022)

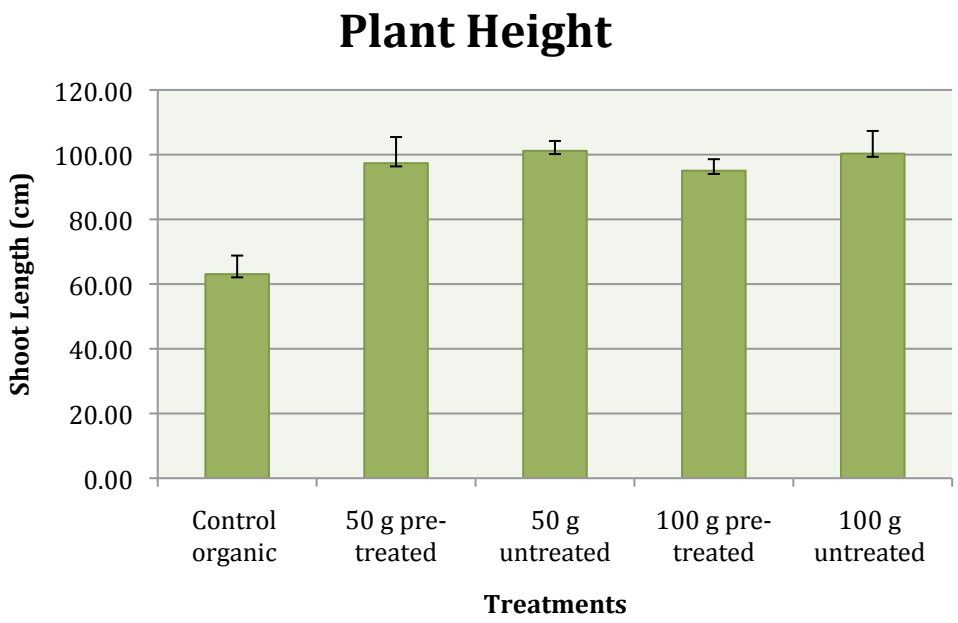

Fig. 2.5b – Pre-treated and Untreated Biomass Biofertilizer Application in Organic Potting Soil and its Effects on Plant Height (P=0.0044)

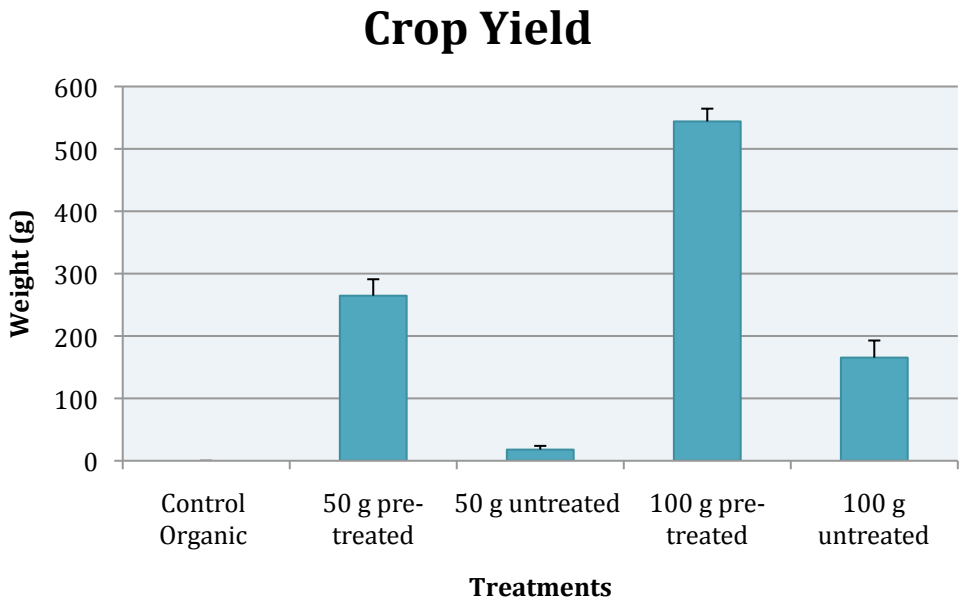

Fig. 2.5c – Pre-treated and Untreated Biofertilizer Application in Organic Potting Soil and its Effects on Crop Yield (P=0.0004)

When comparing the biofertilizer effects on both synthetic and organic potting soils, it can be concluded that the 50 g and 100 g biofertilizer treatments applied to the synthetic potting soil (Table 1) performed better than those mixed with the organic potting mix (Table 2) as presumed, given that the synthetic medium had a greater concentration of added nutrients at the beginning.

Table 1 – Effects of Biofertilizer Incorporated in Synthetic Potting Soil on Tomato Plant Growth Parameters. Values are Mean ± Standard Error

Growth Parameters - Synthetic			
Treatments	Number of Flowers	Plant Height (cm)	Fresh Fruit Weight (g)
Control	14.7 ± 1.856	66.46 ± 3.05	92.67 ± 6.330
50 g pre-treated	37 ± 6.083	92.08 ± 4.93	305.23 ± 25.039
100 g pre-treated	35 ± 3.464	96.10 ± 4.71	421.10 ± 64.936

Table 2 – Effects of Pre-treated and Untreated Biofertilizer on Organic Potting Soil on Tomato Plant Development. Values are Mean ± Standard Error

Growth Parameters - Organic			
Treatments	Number of Flowers	Plant Height (cm)	Fresh Fruit Weight (g)
Control	1 ± 0.577	63.07 ± 5.734	0 ± 0.000
50 g pre-treated	8 ± 1.155	97.37 ± 8.077	88.2 ± 26.401
50 g untreated	5.7 ± 0.667	101.18 ± 3.053	5.97 ± 5.967
100 g pre-treated	3 ± 0.577	95.04 ± 3.559	181.33 ± 20.461
100 g untreated	13 ± 3.055	100.33 ± 6.995	55.13 ± 27.398

Fig. 2.6 – Delay in Biofertilizer Availability for the Plants Uptake Led to Early Signs of Nutrient Deficiency

A second biofertilizer experiment was conducted after observing a delay in the biofertilizer uptake (Fig. 2.6) to investigate if earlier biofertilizer application into standard potting soil (peatmoss: vermiculite: perlite) might result in an improved plant response. The results illustrated a significant difference between the 50 g and 100 g biofertilizer treatments applied 22 days prior to transplant compared to those applied at the time of transplant (Fig. 2.7). The number of flowers (Fig. 2.8a), early fruit development (Fig. 2.8b), and the number of branches (Fig. 2.8c), were all significantly greater in the treatments applied 22 days prior to transplant as

compared to the control group and biofertilizer treatments applied at the time of transplant.

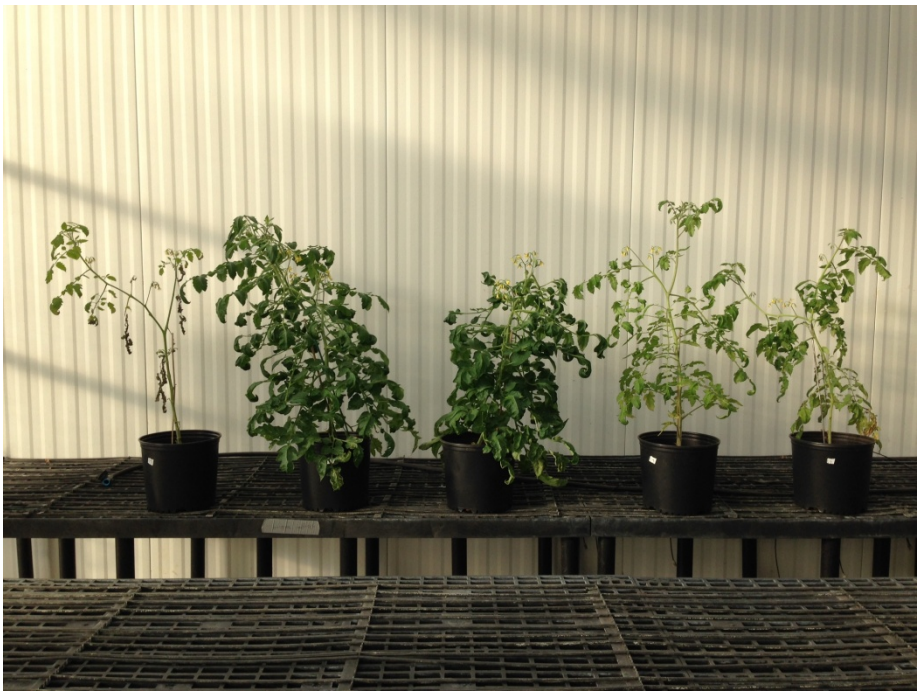

Fig. 2.7 – Effects of the Timing of Biofertilizer Incorporation into Potting Soil. Control, 50 g 22 Days Prior, 100 g 22 Days Prior, 50 g at Time of Transplant, 100 g at Time of Transplant (From Left to Right)

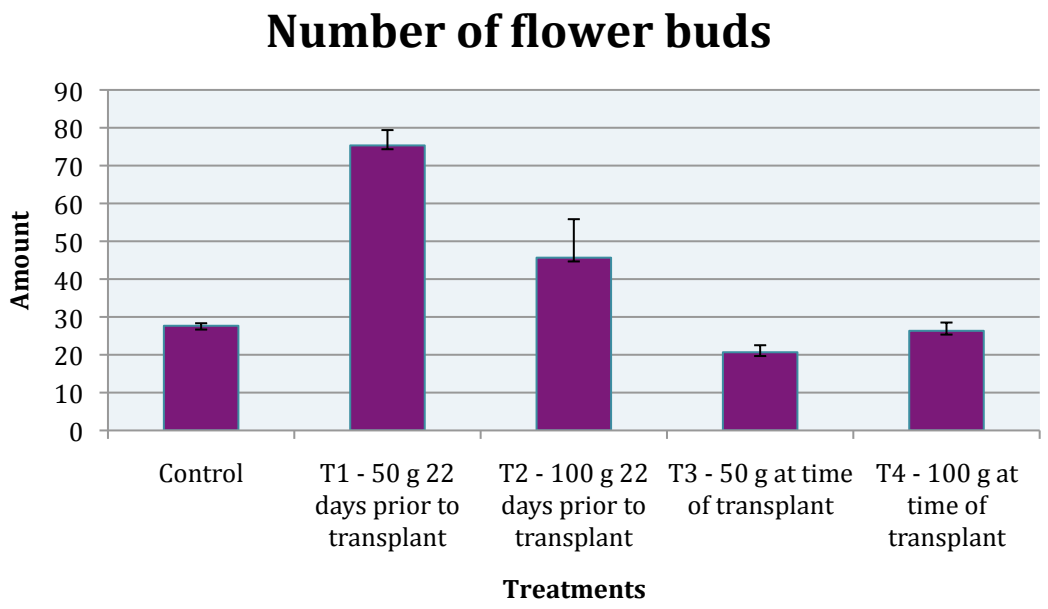

Fig. 2.8a – Effects of the Timing of Biofertilizer Incorporation into Potting Soil on Flower Development (*P*=0.0001)

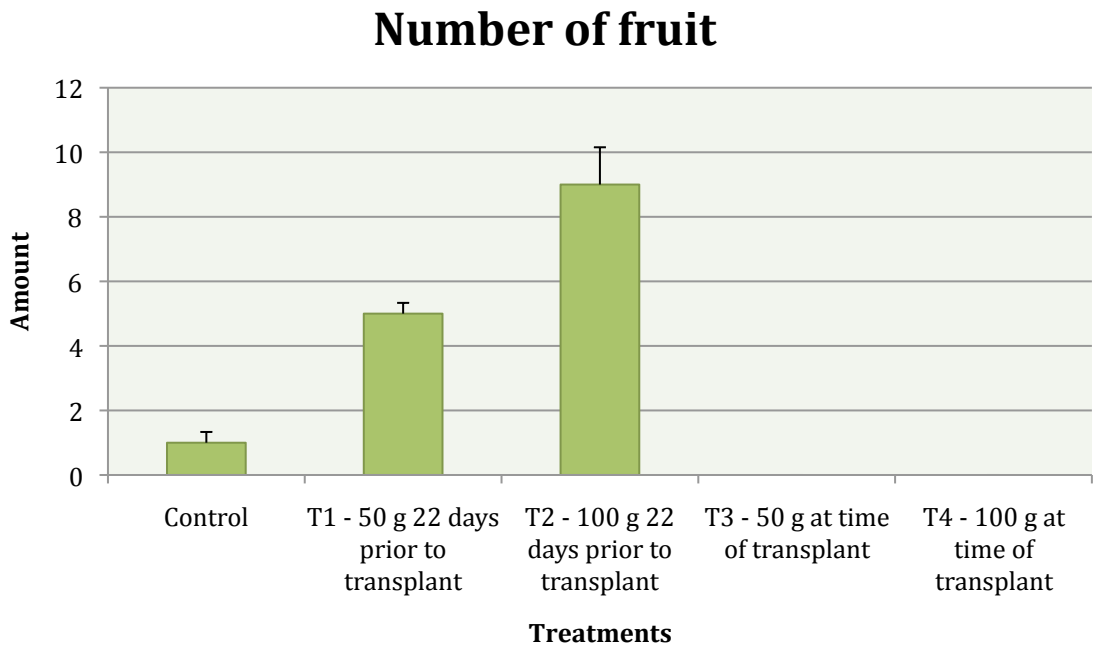

Fig. 2.8b – Effects of the Timing of Biofertilizer Incorporation into Potting Soil on Fruit Development (*P*=0.0129)

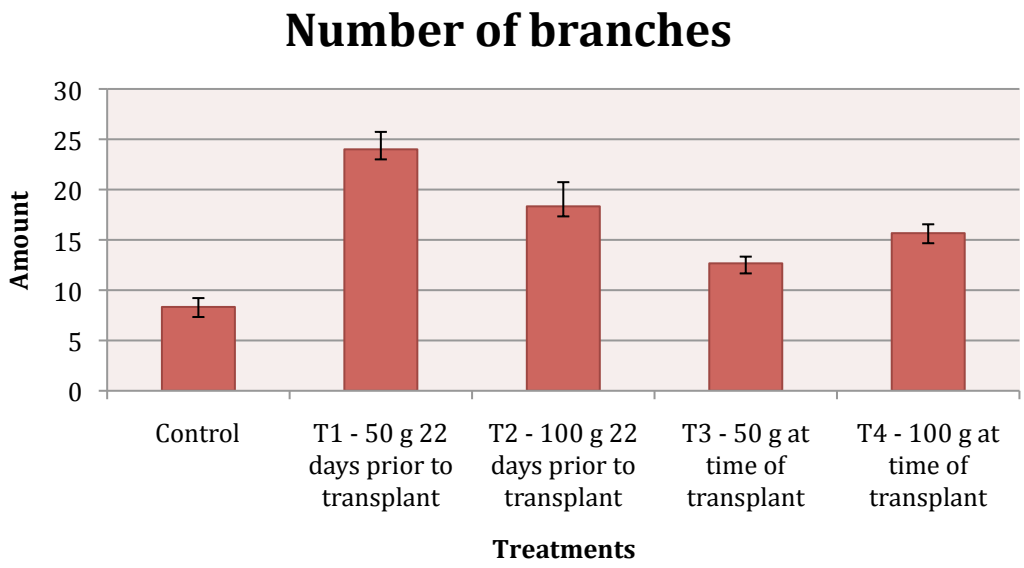

Fig. 2.8c – Effects of the Timing of Biofertilizer Incorporation into Potting Soil on the Number of Branches Per Plant (*P*=0.0002)

One sample was taken at random from each treatment to measure total fresh plant weight (g). The biofertilizer treatments applied 22 days prior to transplant yielded higher biomass accumulation with greater shoot and root fresh weights (g) (Fig. 2.9). Between the two biofertilizer treatments applied 22 days prior to transplant, the 100 g treatment resulted in higher fruit development and greater total fresh plant weight (g), whereas the 50 g treatment resulted in higher number of branches and greater flower development per plant. However, when average plant height was analyzed there was minimal variation between the control group and all biofertilizer treatments. In fact, both biofertilizer treatments applied at the

time of transplant had higher plant heights (cm) (Fig. 2.10), but much lower overall biomass accumulation and productivity (Table 3).

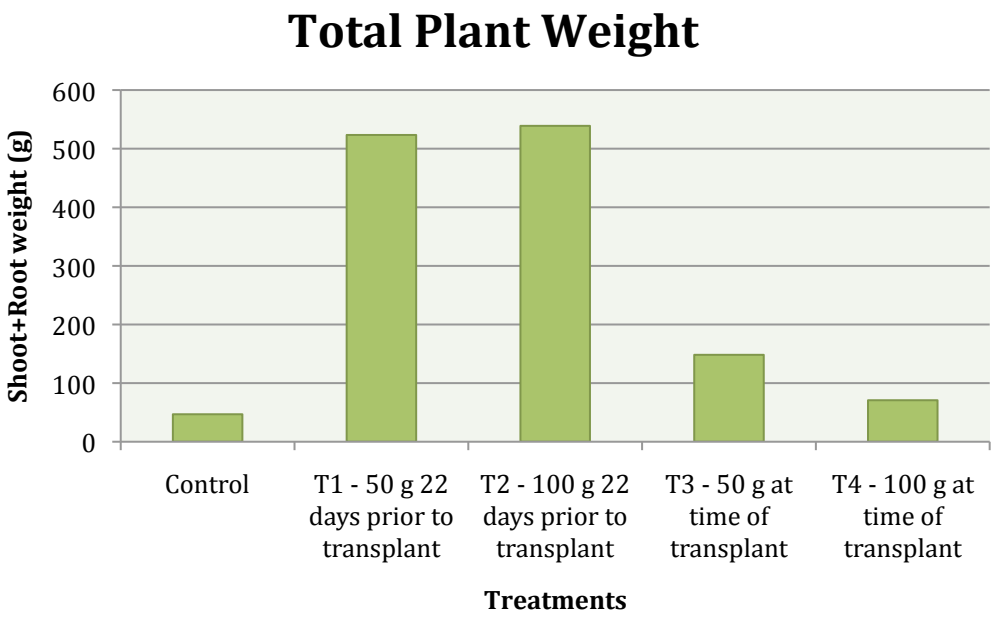

Fig. 2.9 – Total Fresh Shoot + Root Weight g of Samples Selected at Random From the Various Treatments

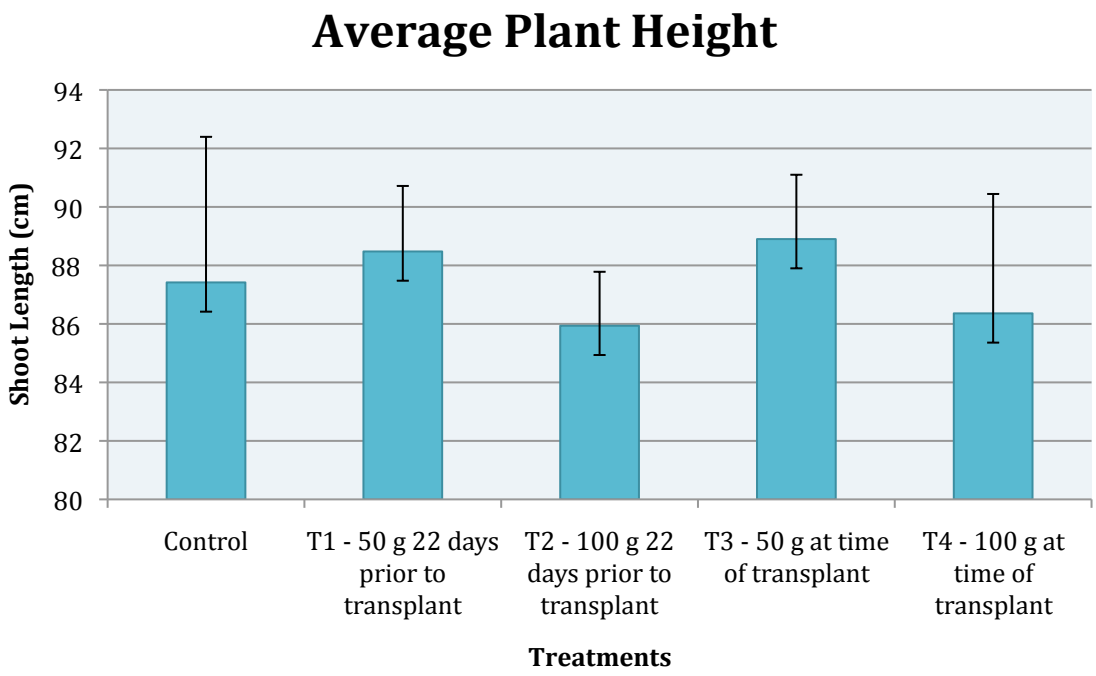

Fig. 2.10 – Effects of the Timing of Biofertilizer Application on Shoot Length (cm) of Tomato Plants

Table 3 – Effects of the Timing of Biofertilizer Application on The Overall Growth of Tomato Plants

	Growth Parameters			
Treatments	Number of Flowers	Number of Fruit	Number of Branches	Plant Height (cm)
Control	27.67 ± 0.667	0.33 ± 0.333	8.33 ± 0.882	87.42 ± 4.978
T_1 - 50 g 22 days prior to transplant	75.33 ± 4.055	1.67 ± 0.333	24 ± 1.732	88.48 ± 2.240
T_2 - 100 g 22 days prior to transplant	45.67 ± 10.171	3 ± 1.155	18.33 ± 2.404	85.94 ± 1.845
T_3 - 50 g at time of transplant	20.67 ± 1.856	0 ± 0.000	12.67 ± 0.667	88.9 ± 2.200
T_4 - 100 g at time of transplant	26.33 ± 2.186	0 ± 0.000	15.67 ± 0.882	86.36 ± 4.082

2.4. Conclusion

From this investigation it can be concluded that dry *Scenedesmus dimorphus* biomass applied as a biofertilizer to tomato plants, not only enhanced plant height, but also led to greater crop production. Earlier biofertilizer application (weeks prior to transplant) significantly enhanced plant growth – thus suggesting that earlier application is essential for the biomass to be degraded or broken down in order for the nutrients to be more readily available for plant uptake. An application of 50 g per plant was sufficient to be both highly productive and potentially feasible from an economic standpoint. Further studies should be conducted to observe how the *Scenedesmus dimorphus* biomass will react under field conditions, and whether lower application quantities will have similar effects in order to provide the ideal biofertilizer applications for different plant species. It should be noted that although biofertilizers are derived from natural sources and should be less harmful to the environment than synthetic chemicals, ecological toxicology studies should be conducted to assess whether they have any detrimental effects on the ecosystem. It should also be noted that if they are mismanaged and overused, as we have seen with synthetic agrochemicals, the ecological impacts might be the same. Nonetheless, microalgae are an enormous untapped resource with great potential in the agriculture sector and more research needs to be conducted to discover and exploit their potential.

CHAPTER 3

EFFECTS OF AQUEOUS EXTRACTS OF THE MICROALGA *SCENEDESMUS DIMORPHUS* ON GROWTH OF ROMA TOMATO PLANTS

Abstract

Aqueous extracts of *Scenedesmus dimorphus* were applied as biostimulants to study their effects on seed germination and growth of tomato plants (*Solanum lycopersicum* var. Roma). *Scenedesmus dimorphus* culture, growth medium, and different cell extract concentrations (1%, 5%, 10%, 25%, 50%, 75%, and 100%) were used as seed primers to assess germination rate and seedling development. The *Scenedesmus* culture, growth medium, and 50 % and 100 % aqueous extract concentration treatments triggered faster seed germination compared to the control group. Extract concentrations higher than 50 % (0.75 g ml^{-1}) led to shorter root and shoot lengths and lower numbers of lateral roots. The cell extracts were also applied as foliar fertilizers at various concentrations (10%, 25%, 50%, 75%, and 100%) on tomato seedlings. Foliar application of the aqueous extracts at 50 % (3.75 g ml^{-1}) enhanced plant height as well as the number of flowers and branches per plant. Higher extract foliar concentrations decreased plant growth and development.

3.1. Introduction

In the search for natural products certain microalgae species (i.e. *Dunaliella* sp., *Haematococcus* sp.) have received more attention due to their ability to produce large amounts of functional products such as antioxidants, carotenoids, polyunsaturated fatty acids, and other potential functional food ingredients

(Herrero, Cifuentes & Ibanez, 2006; Plaza et al., 2010; Rodríguez-Meizoso et al., 2010). Lesser attention has been placed on the potential agricultural applications of microalgae. Microalgae are a large and extremely diverse group of organisms with huge potential for discovery of new bioactive compounds that can be utilized not only within the food industry but also in the agricultural sector. Earlier studies investigating the effects of algae extracts on seed germination observed faster germination and greater growth of rice seeds that were treated with extracts (Shukla & Gupta, 1967). More recent studies have obtained similar results utilizing seaweed extracts on tomato and wheat seeds, although they have also observed a growth inhibition with increasing extract concentrations (Hernández-Herrera, Santacruz-Ruvalcaba, Ruiz-López, Norrie & Hernández-Carmona, 2013; Kumar & Sahoo, 2011; Kumari, Kaur & Bhatnagar, 2011). The growth medium from several microalgae species has been shown to contain phytohormones – gibberellin and cytokinin, which are known to play crucial roles in plant development (Burkiewicz, 1987; Tarakhovskaya, Maslov & Shishova, 2007).

Several studies utilizing seaweed extracts as foliar sprays have observed an increase in plant biomass accumulation and greater crop yields with foliar applications (Hernández-Herrera et al., 2013; Kumari et al., 2011).The present investigation was conducted to analyze whether *Scenedesmus dimorphus* culture, its growth medium, and cellular extracts would have any effects on tomato seed germination and plant growth.

3.2. Materials and Methods

Cultivation and harvesting

The microalga *Scenedesmus dimorphus* was cultivated outdoors in seven 4'x 48' production row panel photobioreactors (Fig. 3.1) using standard BG-11 algae culture medium (Stanier et al., 1971), bubbled with air mixed with 1% carbon dioxide, at the Arizona State University, Arizona Center for Algae Technology and Innovation (AzCATI). Samples were taken to analyze the biomass biochemical composition. The biomass was stressed for high lipid production and was harvested by centrifugation at day 14. The biomass was then frozen until further use. The frozen biomass was thawed at 4^{o}C for 24 hours. Once thawed, the biomass was spread onto ten metal trays to a thickness of 1.5 cm and placed inside a Freeze-dryer (Millrock Max Series) at -40^{o}C and allowed to dry for approximately 48 hours. The dried biomass was then collected and stored in a cold room at 4^{o}C until further use.

Fig. 3.1 – Early Growth Phase of *Scenedesmus dimorphus* Cultivation in 4' x 48' Panel Photobioreactors

Chemical composition

Biomass samples were sent to the Arizona State University Goldwater Analytical Laboratory to analyze the biomass elemental composition. Nitrogen was analyzed through combustion analysis using a PE 2400 CHN analyzer.

Extraction

One kilogram (kg) of the dried biomass was suspended in distilled (DI) water at a concentration of 150 g L^{-1}. The suspension was then processed through a Microfluidizer processor (M-110EH-30) to lyse the cell wall and obtain the

intracellular extracts. The resulting extract was then centrifuged at 6,000 rpm for ten minutes at 22^{o}C in order to separate the cell liquid extracts from the biomass residue. To minimize potential degradation, the resulting extract suspension was collected in a flask covered with aluminum foil and stored in a cold room at 4^{o}C. The biomass residue was also stored in the cold room for potential future use.

Seed Primer

The cellular extracts, growth medium, and culture of *Scenedesmus dimorphus* were screened to assess their ability to stimulate faster seed germination. The growth medium was obtained by collecting the supernatant after centrifugation (4,000 rpm for 10 min), of two 50 ml *Scenedesmus* culture samples. Each treatment was replicated three times with 10 seeds per replicate. The seeds were surface sterilized with 10 ml of 5 % solution of sodium hypochlorite for 10 min, rinsed twice with DI water and soaked in 10 ml of the respective treatment solutions for 24 hours (Fig. 3.2b). Following the 24-hour priming (soaking) period, the seeds were placed between two 42.5 mm Whatman 1 filter papers and allowed to dry for 24 hours (Fig. 3.2c).

The seeds were then transferred to a sterile 100-mm Petri plate containing a moist 75-mm Whatman 1 filter, soaked with 3 ml of DI water (Fig. 3.2d). The plates were incubated at room temperature at 21^{o}C under a 16 hour light/8 hour dark cycle. Seed germination was checked at 24-hour intervals, over a period of 10 days, and counted as germinated if at least 2 mm of the radicle had emerged; the filter paper for all treatments was saturated as needed with 3 ml of DI water to maintain

moisture. A caliper was used to measure root, shoot, and leaf lengths (mm). Other measured variables included germination percentage (GP) and germination energy (GE). Germination percentage, an estimate of the viability of a population of seeds, was calculated as GP = (number of germinated seeds/total number of seeds) x 100. Germination energy, a measure of the speed of germination and hence assumed as a measure of the vigor of the seedling produced, was calculated according to (Hernández-Herrera et al., 2013), where GE=(number of germinating seeds/number of total seeds per treatment after germination for 3 days) x 100.

Fig. 3.2a-d – a) Seeds Inoculated with *Scenedesmus dimorphus* Culture; b) Soaking Seed Treatments (10 ml); c) 24 Hour Drying Period Between Two Filter Papers; d) Ongoing Germination Experiment over 10 Days.

Treatments:

1) Control: DI water
2) T_1: 1 % concentration (0.1 mL extract in 9.9 mL DI water)
3) T_2: 5 % concentration (0.5 mL extract in 9.5 mL DI water)
4) T_3: 10 % concentration (1 mL extract in 9 mL DI water)
5) T_4: 25 % concentration (2.5 mL extract in 7.5 mL DI water)
6) T_5: 50 % concentration (5 mL extract in 5 mL DI water)
7) T_6: 75 % concentration (7.5 mL extract in 2.5 mL DI water)
8) T_7: 100 % concentration (10 ml)
9) T_8: *Scenedesmus* growth medium (10 ml)
10) T_9: *Scenedesmus* culture (10 ml)

Foliar spray

The experiment consisted of five extract treatments at various concentrations (10, 25, 50, 75 and 100%) and a control group (DI water) with three replicates per treatment (Fig. 3.3b). Each plant received two sprayings; the first (50 ml) was applied at the time of transplant, and the second (100 ml) four weeks later. The experiment was performed under greenhouse conditions with treatments arranged in a complete randomized block design. During foliar treatment applications, the pot surface was completely covered with aluminum foil to prevent spray run-off from coming into contact with the potting soil and potentially be taken up by the roots (Fig. 3.3a). The sprays were conducted in the morning when the stomata are open due to water pressure allowing greater foliar penetration. All

plants were watered as needed throughout the experiment, except after foliar application when they were not watered for 24 hours.

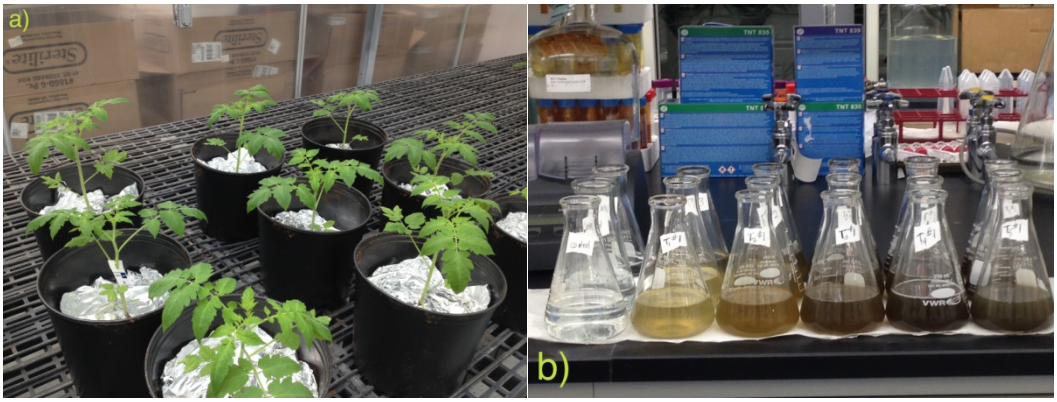

Fig. 3.3a-b – a) Pots Covered with Aluminum Foil to Inhibit Foliar Spray to Come in Contact with Soil; b) Foliar Treatment Solutions at Various Concentration – Darker Pigmentation = Greater Extract Concentration

Treatments:

**for 50 ml spray treatments the concentrations below were reduced in half to total 50 ml volume*

1) Control (DI water) 100 ml

2) T_1: 10 % (v/v) 10 mL extract in 90 mL DI water

3) T_2: 25 % (v/v) 25 mL extract in 75 mL DI water

4) T_3: 50 % (v/v) 50 mL extract in 50 mL DI water8

5) T_4: 75 % (v/v) 75 mL extract in 25 mL DI water

6) T_5: 100 % (v/v) 100 ml extract

Statistical analysis

Statistical analyses were conducted using StatPlus®:mac LE programming (AnalystSoft, Inc.). All experiments were analyzed using a one-way analysis of variance (ANOVA), to test difference among the means: the level of significance was set at $P< 0.05$.

3.3. Results and Discussion

Chemical composition

The biochemical composition of the biomass contained 40% lipids, 30% carbohydrates and 23% protein. CHN analyses demonstrated that the biomass contained 2.53 % nitrogen per gram, meaning that per 50 g and 100 g biofertilizer application there was about 1.25 g and 2.5 g of nitrogen, respectively.

Seed primer

The *Scenedesmus dimorphus* culture, growth medium, 50 % and 100 % extract concentration treatments, triggered faster seed germination – 2 days earlier than the controls. The *Scenedesmus dimorphus* culture treatment (T_9) was the only one to have half of the seeds germinate by the fourth day. By the fifth day most treatments had reached full germination percentage (Fig. 3.4). Most treatments, with the exception of the growth medium (T_8) and the 25 % (0.375 g ml^{-1}) extract concentration (T_4), had seeds that did not germinate. Since the majority of the seeds within such treatments did germinate, it was concluded that some of the seeds were not viable. Germination energy calculations demonstrated a positive relationship

between increasing energy and increasing extract concentration treatments. However, the largest germination energy (63%) was observed on seeds treated with *Scenedesmus dimorphus* culture (Fig. 3.5).

Previous studies conducted on tomato seeds utilizing seaweed extracts at varying concentrations obtained similar results. Hernández-Herrera et al. (2013) noticed that a concentration of 0.009 g ml^{-1} resulted in the highest germination percentage, and greater plant growth, and higher concentrations exhibited a negative effect on seed germination. Similarly, Kumar & Sahoo (2011) observed that seaweed extract concentrations greater than 20 % resulted in smaller root lengths, lower number of lateral roots, and shorter shoot length. The data showed some similarities. For instance, extract concentrations higher than 50 % or 0.75 g ml^{-1} resulted in smaller root lengths and lower number of lateral roots. The tomato seeds inoculated with *Scenedesmus dimorphus* culture led to longer root lengths (Fig. 3.6a), shoot length (Fig. 3.6b), longer leaf lengths (Fig. 3.6c), and greater lateral root development (Fig. 3.6d), outperforming all other treatments. The higher the number of lateral roots the greater the plants ability to acquire water and nutrients, hence seeds inoculated with *Scenedesmus* culture would potentially accumulate greater plant biomass and result in greater crop yields.

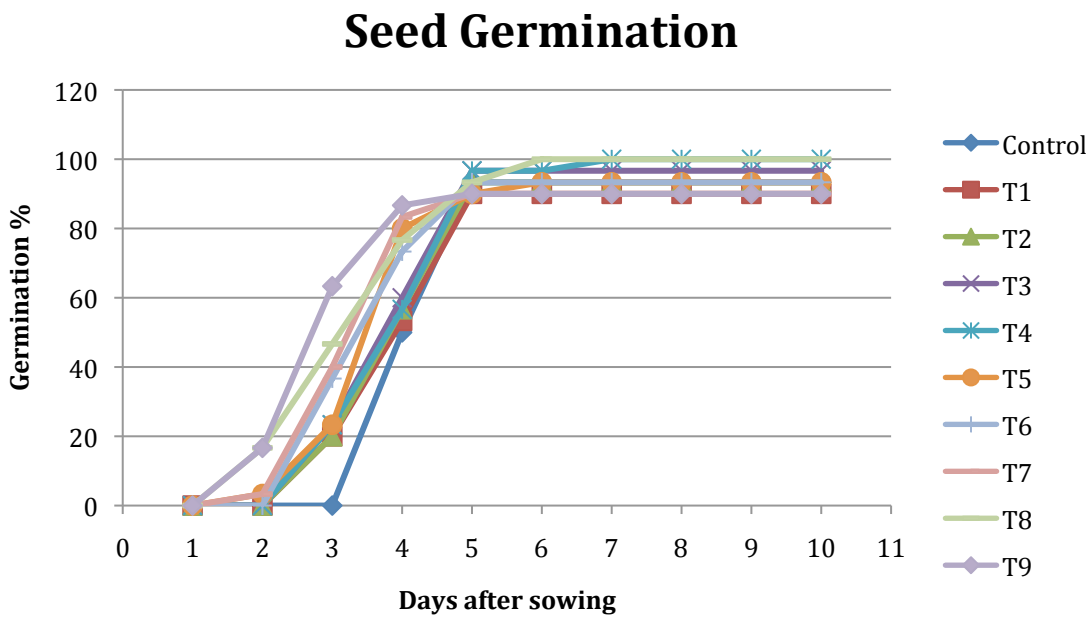

Fig. 3.4 – Seed Germination Percentage: GP = (Number of Germinated Seeds/Total Number of Seeds) x 100

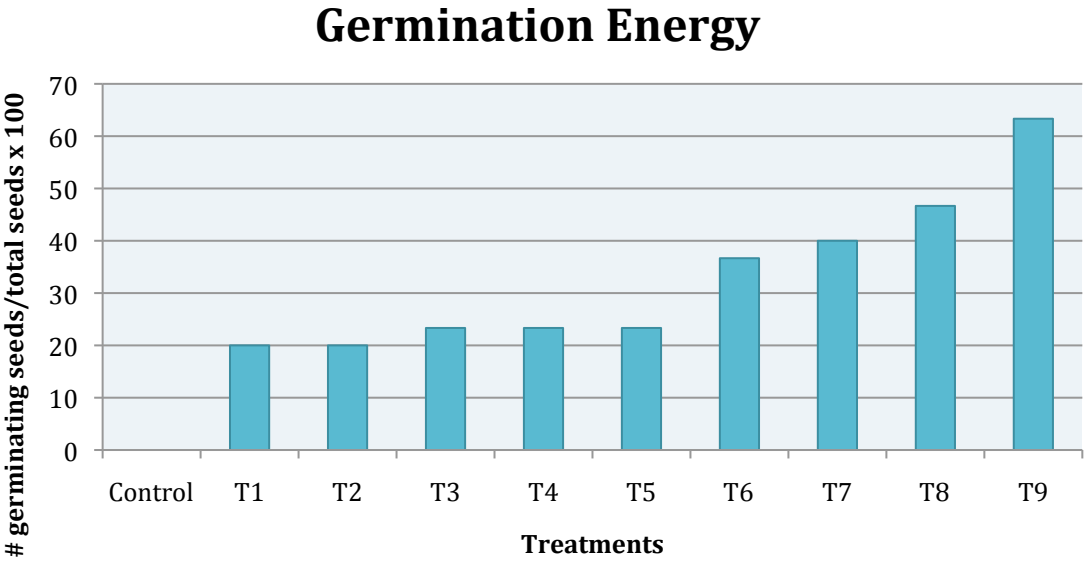

Fig. 3.5 – Germination Energy of Seeds Treated with *Scenedesmus* Culture (T9), Cell Extracts (T1-T7), and Growth Medium (T8)

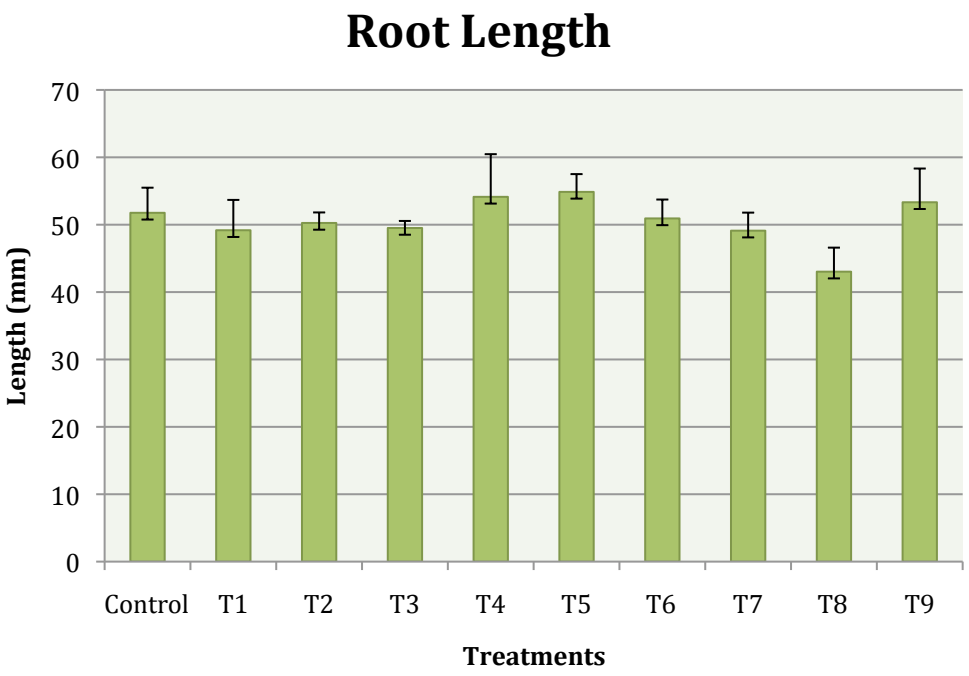

Fig. 3.6a – Effects of *Scenedesmus* Culture, Growth Medium, and Extract Concentration Effects on Root Development of Tomato Seedlings

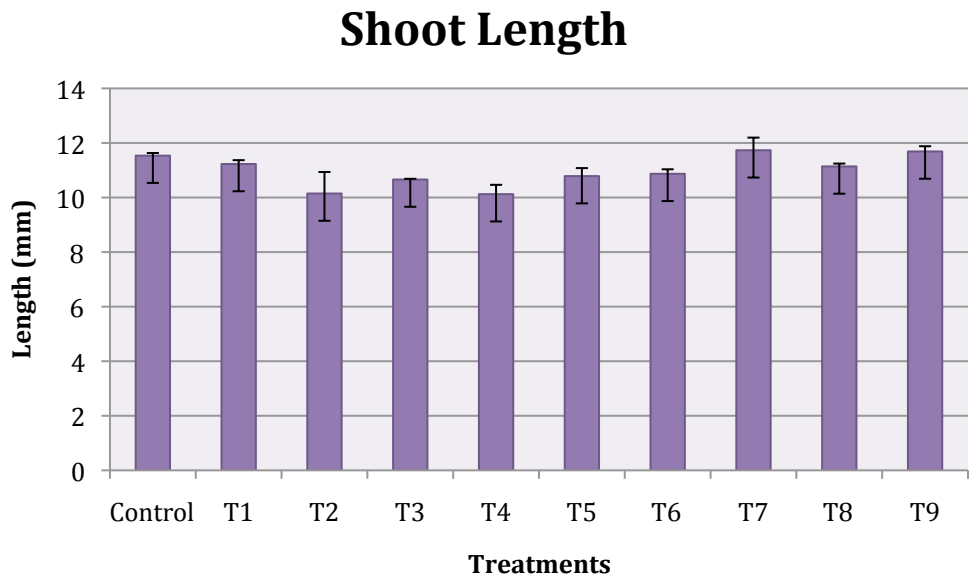

Fig. 3.6b – Effects of *Scenedesmus* Culture, Growth Medium, and Extract Concentration Effects on Shoot Length of Tomato Seedlings

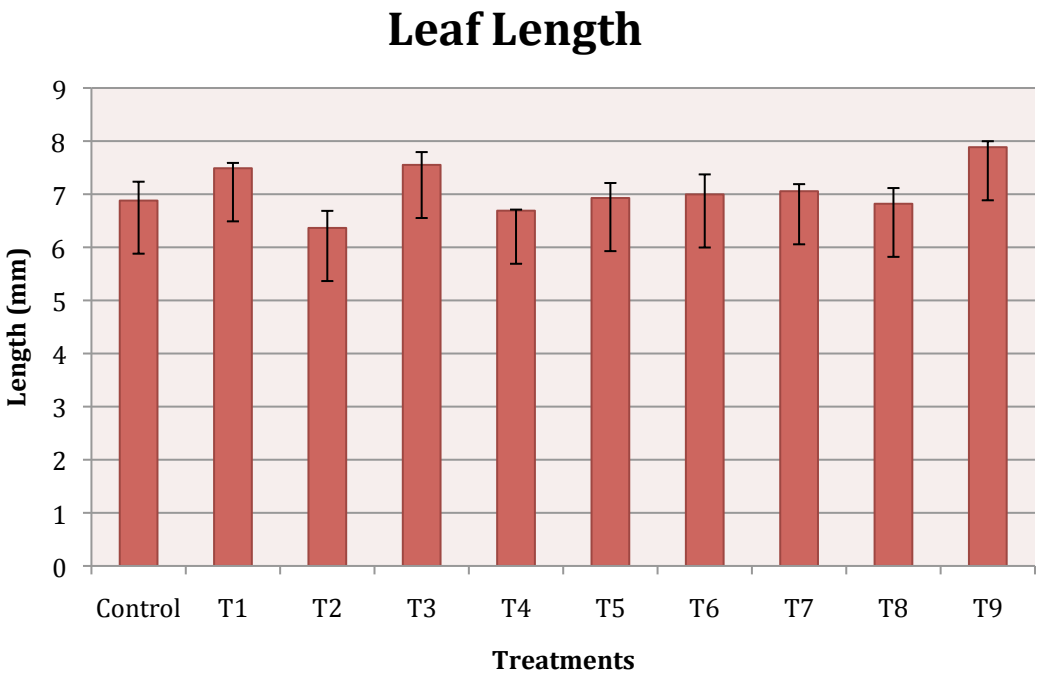

Fig. 3.6c – Effects of *Scenedesmus* Culture, Growth Medium, and Extract Concentration Effects on Leaf Length of Tomato Seedlings

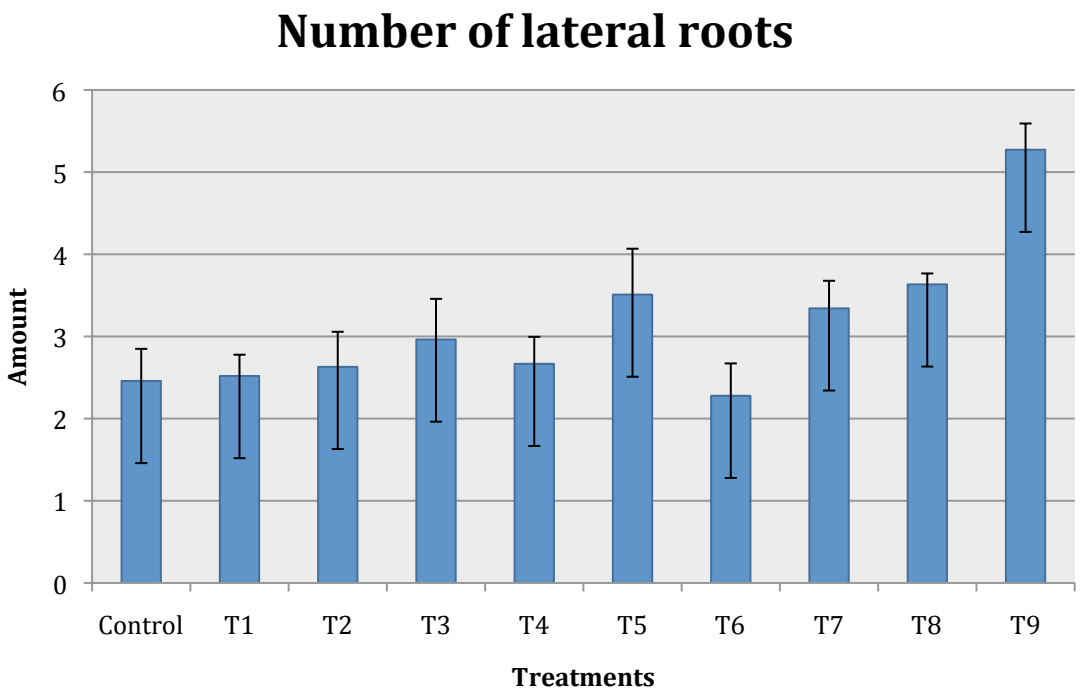

Fig. 3.6d – Effects of *Scenedesmus* Culture, Growth Medium, and Extract Concentration on Lateral Root Development of Tomato Seedlings

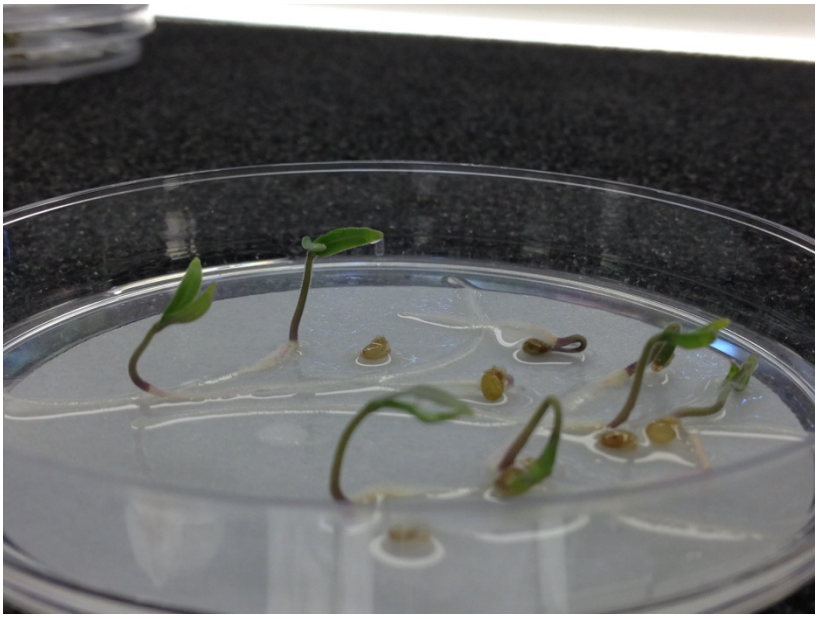

Fig. 3.7 – Seedling Growth at 5 Days After Treatment with *Scenedesmus* Extracts

Foliar spray

The 50 % or 3.75 g ml^{-1} foliar spray, 7.5 g ml^{-1} for 100 ml second application, led to greater number of flowers (Fig. 3.8a), branches (Fig. 3.8b), and the greatest plant height (Fig. 3.8c). Foliar sprays of higher concentrations resulted in less flower development, lower number of branches, and a slight decrease in plant height (Table 4). These results are similar to those obtained by (Hernández-Herrera et al., 2013), who observed smaller shoot lengths on foliar sprays of seaweed extracts at concentrations greater than 0.18 g ml^{-1} (0.4 %), while Kumari, Kaur & Bhatnagar (2011) observed the opposite, with greater plant growth occurring with increasing concentrations of seaweed extracts. One sample per treatment (Fig. 3.10) was chosen at random in order to measure total fresh plant weight (g). The 75 % or 5.625 g ml^{-1}, 11.25 g ml^{-1} for the 100 ml spray treatment, led to greater total fresh plant weight (g), with a margin of approximately 9 grams over the 50 % or 3.75 g ml^{-1} foliar treatment (Fig. 3.9).

Foliar sprays provide a more rapid nutrient utilization and enable correction of deficiencies compared to soil fertilizer applications. The greatest difficulty in supplying nutrients via foliar sprays is in adequately applying the right quantity the without burning the leaves. Although, the results indicated a positive correlation between foliar extract applications and greater plant growth, for foliar sprays to gain a greater acceptance for application in crop production, further studies need to be conducted since there are a plethora of factors (temperature, humidity, light intensity, nutrient concentration, surfactants, application rate, etc.) that play a role

in the efficiency of foliar applications (Fernández & Eichert, 2009; Haynes & Goh, 1977; Kannan & Charnel, 1986).

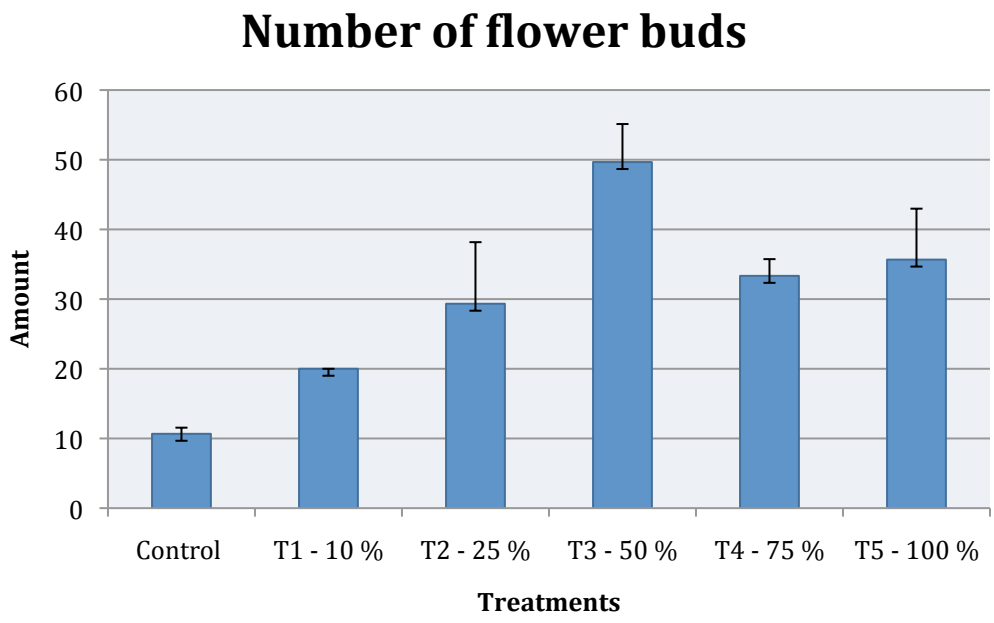

Fig. 3.8a – Effects of Cell Extracts as Foliar Treatments on Flower Development on Tomato Plants

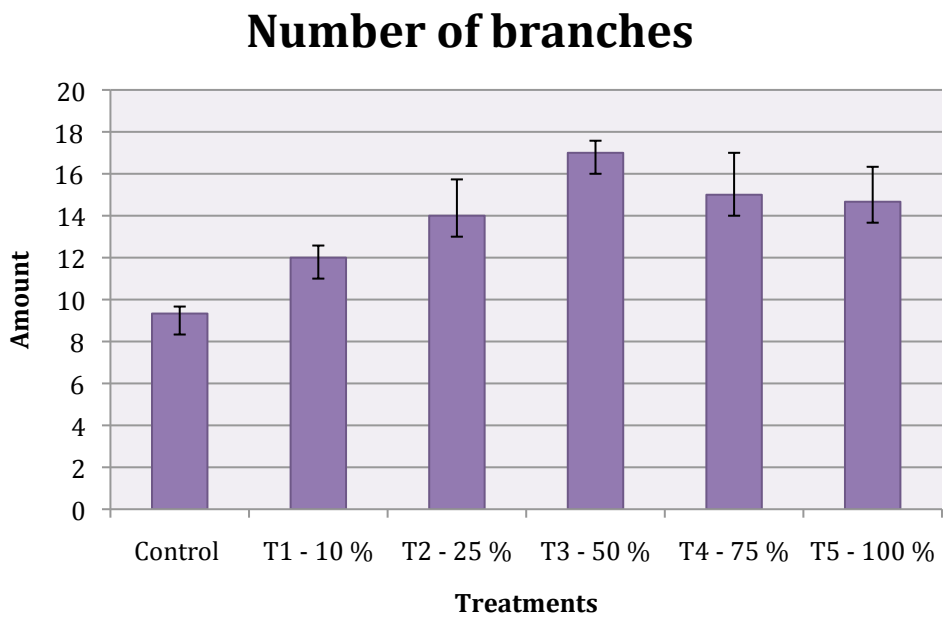

Fig. 3.8b – Effects of Cell Extracts as Foliar Treatments on Average Number of Branches on Tomato Plants

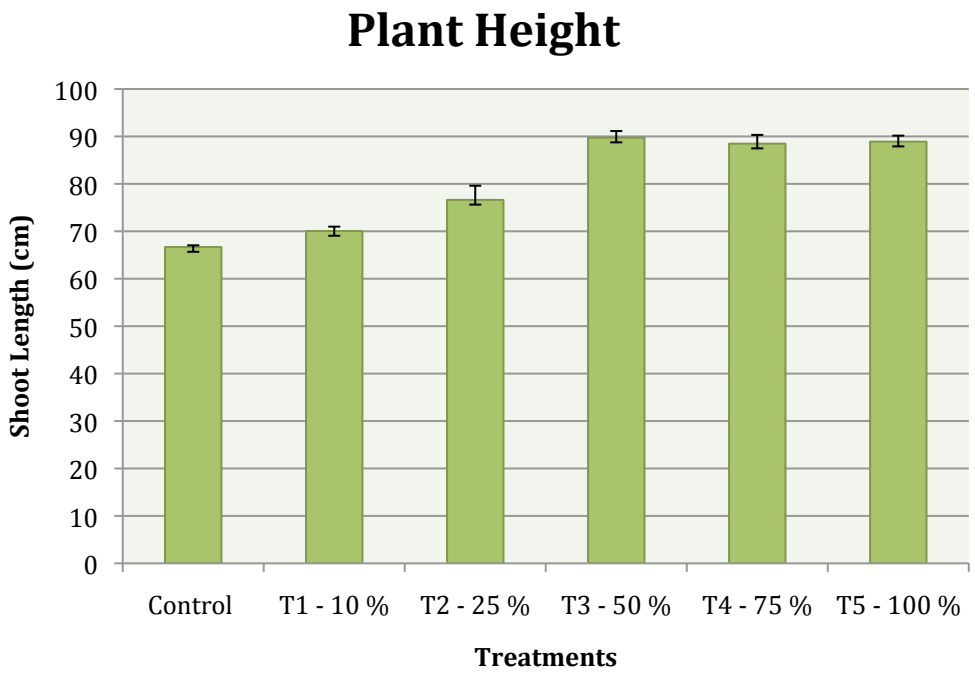

Fig. 3.8c – Effects of Cell Extracts as Foliar Sprays on Average Shoot Length Per Treatment on Tomato Plants

Table 4 – Effects of Extract Foliar Sprays on Growth Parameters of Tomato Plants

Treatments	Concentrations	Growth Parameters			
		Number of Flowers	Number of Branches	Number of Fruit	Plant Height (cm)
Control	0 (g/ml)	10.67 ± 0.882	9.33 ± 0.333	0 ± 0.000	66.68 ± 0.367
T_1 - 10 %	0.75 (g/ml)	20.00 ± 0.000	12 ± 0.577	0 ± 0.000	70.06 ± 0.923
T_2 - 25 %	1.875 (g/ml)	29.33 ± 8.838	14 ± 1.732	0.67 ±0.667	76.62 ± 2.986
T_3 - 50 %	3.75 (g/ml)	49.67 ± 5.457	17 ± 0.577	0.33 ± 0.333	89.75 ± 1.388
T_4 - 75 %	5.625 (g/ml)	33.33 ± 2.404	15 ± 2.000	0.33 ± 0.333	88.48 ± 1.845
T_5 - 100 %	7.5 (g/ml)	35.67 ± 7.311	14.67 ± 1.667	0.33 ± 0.333	88.90 ± 1.270

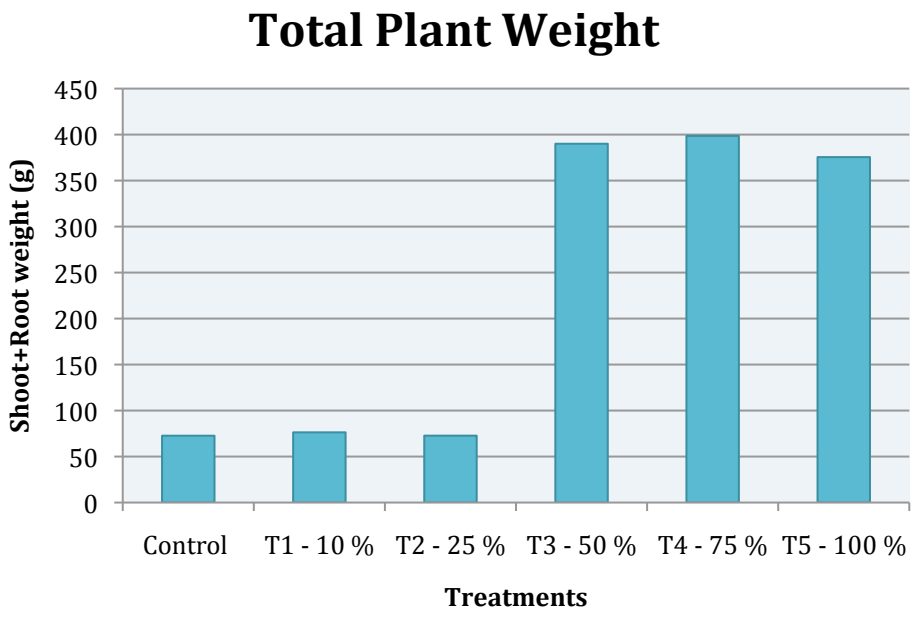

Fig. 3.9 – Effects of *Scenedesmus dimorphus* Extracts as Foliar Sprays on Total Fresh Weight of Tomato Plants (One Sample Per Treatment Chosen at Random)

Fig. 3.10 – Effects of Foliar Sprays on Tomato Plant Growth: (From Left to Right), Control; 10 % Conc.; 25% Conc.; 50 % Conc.; 75 % Conc.; and 100 % Extract Concentration.

3.4. Conclusion

Seed and foliar application of *Scenedesmus dimorphus* aqueous extracts and growth medium had a major positive affect on seed germination and plant growth. However, like with synthetic agrochemicals, there appears to be a cutoff concentration, since higher extract concentrations led to a decrease in the overall growth and development of tomato plants. This suggests that *Scenedesmus dimorphus* may produce some sort of plant growth hormone since the growth medium application alone resulted in positive growth effects when applied as a seed primer on tomato seeds. Seed inoculation with *Scenedesmus dimorphus* culture had the greatest effects on seed germination and seedling growth. Further studies on the

applications of microalgae culture, growth medium and cell extracts on different plant species are needed. Chemical characterization of the extracts and growth medium would enable us to see if there are in fact phytohormones present within the extracts. Nonetheless, this study demonstrates that there are positive correlations between aqueous foliar sprays and growth of tomato plants. Additional studies on floral crops should be conducted since foliar sprays are commonly utilized within the floriculture industry.

CHAPTER 4

SCREENING FOR ANTIMICROBIAL ACTIVITY OF THE MICROALGA *SCENEDESMUS DIMORPHUS*

Abstract

Cellular aqueous extracts of *Scenedesmus dimorphus* at various dosages (50 ul, 100 ul and 200 ul) as well as extract residue re-suspended in 20 ml of methanol and ethanol were assayed for potential antimicrobial activity against a bacterium, and two fungi using a disc-diffusion bioassay. Each plate contained a negative control (extract solvent), positive control (antimicrobial), and three other discs (6 mm) that had been impregnated with different extract dosages. From this initial screening, none of the cellular extracts demonstrated antimicrobial activity against the selected microorganisms.

4.1. Introduction

One potential application from microalgal by-products is in the arena of pharmaceuticals. Microalgae have been known to produce bioactive compounds able to act as toxic chemicals to other algae as well as other organisms (Borowitzka, 1995; Cannell, Owsianka & Walker, 1988; Kellam & Walker, 1989; Metting & Pyne, 1986). Several studies have demonstrated the capability of different *Scenedesmus* strains to inhibit the growth of microorganisms (Catarina Guedes, Barbosa, Amaro, Pereira & Xavier Malcata, 2011; Nair & Krishnika, 2011; Öztürk, Asl & Beyatl, 2006). The present study investigated the potential antimicrobial applications of the cellular extracts of the microalga *Scenedesmus dimorphus* on the Gram-negative

bacterium, *Escherichia coli* ATCC 25922; the fungi, *Candida albicans* ATCC 90028; and *Aspergillus brasiliensis* ATCC 16404.

4.2. Materials and Methods

Cultivation and harvesting

The microalga *Scenedesmus dimorphus* was cultivated outdoors in seven 4'x 48' production row panel photobioreactors (Fig. 4.1) using standard BG-11 algae culture medium (Stanier et al., 1971), bubbled with air mixed with 1% carbon dioxide, at the Arizona State University, Arizona Center for Algae Technology and Innovation (AzCATI). Samples were taken to analyze the biomass biochemical composition. The biomass was stressed for high lipid production and was harvested by centrifugation at day 14. The biomass was then frozen until further use. The frozen biomass was thawed at 4^{o}C for 24 hours. Once thawed, the biomass was spread onto ten metal trays to a thickness of 1.5 cm and placed inside a Freeze-dryer (Millrock Max Series) at -40^{o}C and allowed to dry for approximately 48 hours. The dried biomass was then collected and stored in a cold room at 4^{o}C until further use.

Fig. 4.1 – Early Growth Phase of *Scenedesmus dimorphus* Cultivation in 4' x 48' Panel Photobioreactors

Chemical composition

Biomass samples were sent to the Arizona State University Goldwater Analytical Laboratory to analyze the biomass elemental composition. Nitrogen was analyzed through combustion analysis using a PE 2400 CHN analyzer.

Extraction

One kilogram (kg) of the dried biomass was suspended in distilled (DI) water at a concentration of 150 g L^{-1}. The suspension was then processed through a Microfluidizer processor (M-110EH-30) to lyse the cell wall and obtain the

intracellular extracts. The resulting extract was then centrifuged at 6,000 rpm for ten minutes at 22^{o}C in order to separate the cell extracts from the biomass residue. To minimize potential degradation, the resulting extract suspension was collected in a flask covered with aluminum foil and stored in a cold room at 4^{o}C. The biomass residue was also stored in the cold room for potential future use.

GC-MS analysis

Two 50 ml samples of the aqueous extract were frozen at -80^{o}C for 24 hours, and freeze-dried at -40^{o}C to remove the water. The residues were then suspended in 20 ml of either methanol or ethanol. The samples were then sent to the Goldwater Analytical Laboratory to conduct GC-MS analysis for volatile compounds.

Antimicrobial assays

Following (CLSI, 2012) testing procedures, the cell extracts were screened against three microorganisms; *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 90028, and *Aspergillus brasiliensis* ATCC 16404, to determine their ability to inhibit microbial growth.

Escherichia coli ATCC 25922 was grown in Mueller – Hinton Agar, after a 16 – 18 hr incubation period, 4 – 5 colonies were transferred to 5 ml of 0.9 % saline solution and vortexed. Once the suspension reached the turbidity of 0.5 McFarland standard, a sterile cotton swab was introduced to the bacterial suspension and used to inoculate the agar plates. The swab was streaked in three directions over the entire surface of the agar plate, rotating approximately 60^{o} each time to obtain

uniform inoculation. Antibiotic blank discs (6 mm) were impregnated with their respective solutions and allowed to dry in an incubator at 37^{o}C for 8 hours (Fig. 4.2). The antibiotic and extract discs were then placed onto the agar plate using sterile forceps. The plates were inverted and placed in an incubator at 37^{o}C for 16 – 18 hours. After incubation, the diameters of the zones of inhibition were measured to the nearest mm using calipers (across the centers of the disks). The same procedure was applied for *Candida albicans* ATCC 90028 and *Aspergillus brasiliensis* ATCC 16404 however they were cultured in Sabouraud Dextrose agar and incubated for 24 – 36 hours at 30^{o}C.

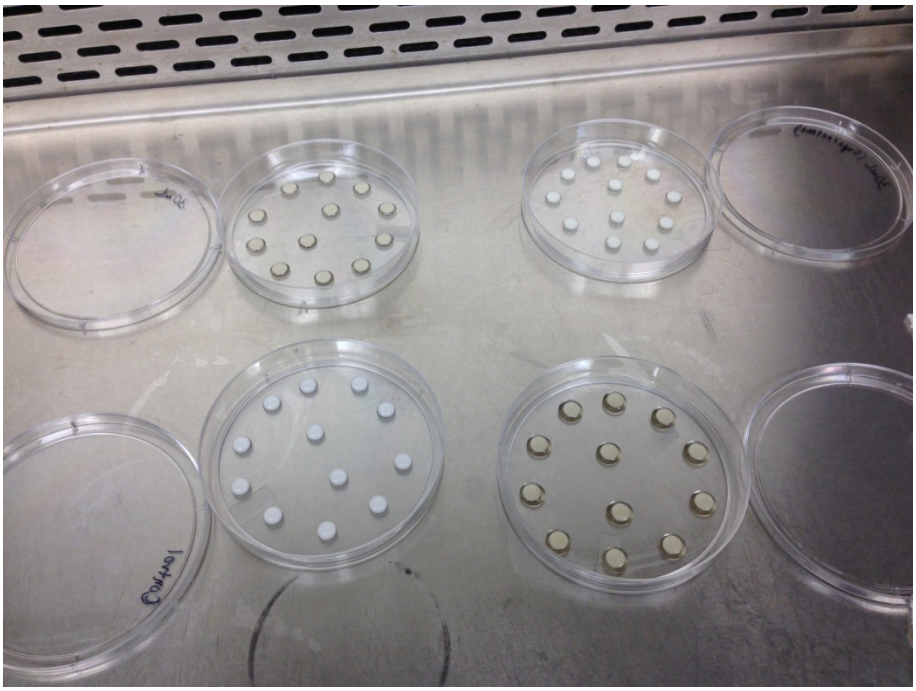

Fig. 4.2 – Blank Discs Impregnated with Different Extract Concentrations

Several trials were conducted to evaluate the extract's potential bioactivity. Each microorganism had three replicates per treatment, and every plate had five treatments: 1) respective antimicrobial depending on microorganism (chloramphenicol – for bacteria and amphotericin B – for yeast and fungi), which were used as positive controls; 2) extract disc (at same dose as antimicrobial); 3) two extract discs at higher dosages (100 and 200 ul); and 4) a negative control – DI water (solvent used for extractions). A second trial was conducted utilizing extracts that had been re-suspended in ethanol or methanol. Two 50 ml samples were taken from the cellular extracts, frozen at -80^{o}C for 24 hours, and freeze-dried, to remove the water. The residues were then suspended in 20 ml of either methanol or ethanol, which were then used to create extract antimicrobial discs (50 ul).

4.3. Results and Discussion

Chemical composition

The biochemical composition of the biomass indicates that it contained 40% lipids, 30% carbohydrates and 23% protein. The CHN analyses demonstrate that the biomass is 2.53 % nitrogen per gram, meaning that per 50 g and 100 g biofertilizer application there was about 1.25 g and 2.5 g of nitrogen, respectively.

GC-MS analysis

In an attempt to identity the compounds responsible for potential antimicrobial activity the samples were re-suspended in 20 ml of methanol and were characterized by GC-MS. Two methanol GC-MS runs were performed, split 1:10

and splitless. The only peak of significance was Oleic acid (Fig. 4.3). The sample might have contained other fatty acids and constituents that are either to dilute to be detected or not sufficiently volatile. Although the re-suspended residue in methanol did contain Oleic acid, it did not inhibit the microbial growth of the selected microorganisms. In previous studies, the antimicrobial activity of macro and microalgae extracts have been attributed to long-chain unsaturated fatty acids including palmitoleic, oleic, linoleic and stearic acids (Plaza et al., 2010; Santoyo et al., 2009).

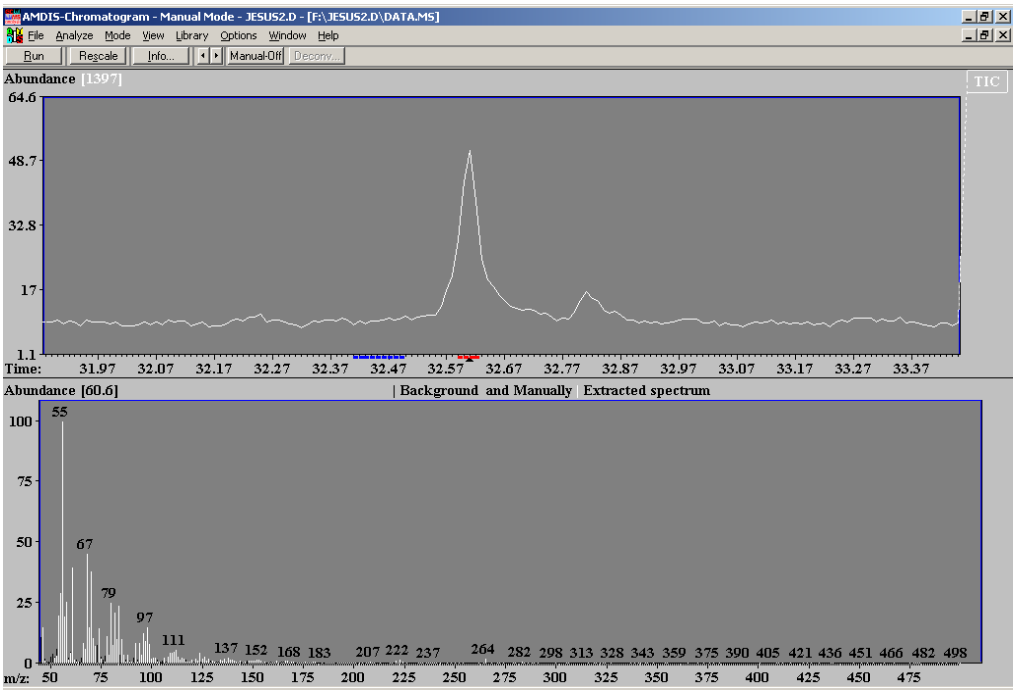

Fig. 4.3 – GC-MS Identification of Compounds Found in the Cell Extracts of *Scenedesmus dimorphus* Showing Oleic Acid Peak

Antimicrobial assay

There was no observed antimicrobial activity from the *Scenedesmus dimorphus* extracts on the selected microorganisms. Neither the extracts diluted in water nor those re-suspended in 20 ml of methanol or ethanol demonstrated any antimicrobial activity. There was a presumption that no bioactivity would be observed on the *Escherichia coli* Petri plates, since previous studies (Bhagavathy, Sumathi, & Jancy Sherene Bell, 2011; Cannell et al., 1988; Debro & Ward, 1979) did not observe inhibition, which has been attributed to the fact that *Escherichia coli* is a Gram-negative microorganism, meaning its cell wall contains two external layers (a thin peptidoglycan membrane and an outer membrane, unique to Gram negative bacteria), making it more difficult for antibiotics to penetrate. However, other microalgae extract studies were able to inhibit the growth of *Escherichia coli* as well as several other Gram-negative organisms (Catarina Guedes et al., 2011; Ibtissam et al., 2009; Kellam & Walker, 1989; Ordog et al., 2004; Öztürk et al., 2006; Plaza et al., 2010; Rodríguez-Meizoso et al., 2010; Santoyo et al., 2009).

Several studies that utilized different *Scenedesmus* sp. strains found antimicrobial activity within their cell extracts against bacteria and fungi (Catarina Guedes et al., 2011; Nair & Krishnika, 2011; Ordog et al., 2004; Öztürk et al., 2006). Nevertheless, it has been suggested that aside from microalga species and culture conditions, other factors such as developmental growth phase, cellular extraction procedure, solvent used for extraction and extraction temperature may play an important role in the bioactivity of the microalgae extracts. The microalgae should be cultured under different conditions, harvested at various growth phases, and

extracted utilizing a variety of methods (Lee, Yoo, Jun, Ahn, & Oh, 2010; Prabakaran & Ravindran, 2011), to fully assess whether bioactive compounds are present. Reviews on the bioactivity of microalgae extracts have been conducted previously (Borowitzka, 1995; Metting & Pyne, 1986; Parsaeimehr & Chen, 2013). Despite the numerous reports and studies on the bioactivity of macro and microalgae extracts, the chemical nature of most extracts remains largely unknown. In only a few isolated cases such as Mason et al., (1982) who were able to isolate and characterize cyanobacterin ($C_{23}H_{23}O_6Cl$) from the cyanobacterium *Scytonema hofmanni*, and Pratt et al., (1944) who reported antimicrobial activity from microalga extracts of *Chlorella* sp. (chlorellin), have microalgae been shown to exhibit antimicrobial activity.

4.4. Conclusion

The initial screening of the aqueous cellular extracts of the microalga *Scenedesmus dimorphus* did not demonstrate any antimicrobial activity against the selected microorganisms selected for testing. Further evaluation using additional extraction methods and solvents should be performed to assess the potential of *Scenedesmus dimorphus* as a source of antimicrobial activity.

CHAPTER 5

CONCLUSION

The present investigation provides further evidence that microalgae biomass and cellular extracts, even aqueous extracts, have potential agricultural applications that are capable of replacing or working in unison with synthetic agrochemicals. The dry biomass applied as fertilizer on tomato plants resulted in greater growth and higher product yields. Earlier application of the biofertilizer resulted in greater plant growth and development. This suggests that the microalga biomass may need to be degraded by microorganisms before its nutrients are readily available for the plant's uptake. This should be further investigated in order to determine the best timing of application to avoid delays in nutrient availability for a rooted plant.

The study, although limited to one microalga and applied only to a single crop – tomato, provides evidence that extracts do in fact enhance plant growth and can lead to higher crop yields. It also provided evidence that seed inoculation with *Scenedesmus* culture triggered faster germination and led to more rapid seedling development. The growth medium that the microalga was grown in similarly increased seedling growth and rate of germination. Further studies should be conducted to assess whether *Scenedesmus dimorphus* exudes chemical compounds into the growth culture, as well as, investigate what compounds present within the extracts and growth medium are responsible for the enhancement of tomato plant growth.

Although foliar fertilizers are in the early stages of development for agricultural application, this study demonstrated that microalgal aqueous extracts

did increase fruit production and led to greater plant growth. Additional studies should be conducted using different microalgal species, different extraction techniques, additional extract concentrations, and additional crops and floral species. Treatments should also be applied under different environmental conditions. For foliar sprays to become successful additional research should be conducted to determine how the chemicals penetrate the leaf surface and how they are conveyed throughout the plant. Without knowing how the receiving plant reacts to the foliar applications, foliar biosprays will remain limited in application.

Preliminary studies of the cell extracts of *Scenedesmus dimorphus* did not demonstrate any antimicrobial activity on the selected microorganisms. However, further studies need to be conducted, evaluating different concentrations, extraction methods, and different microorganisms, in order to deduce whether or not the extracts can exhibit any antimicrobial activity.

Although microalgae biomass and cellular extracts have the capability to potentially replace synthetic agrochemicals, their overuse and mismanagement could result in the same ecological degradation that society is currently experiencing. Furthermore, despite the fact that this study only analyzed the applications of the microalga *Scenedesmus dimorphus*, it is evident that based on the extensive literature review, microalgal biomass and by-products may have significant potential for application within the agriculture sector.

REFERENCES

Abd El Moniem, E. A., & Abd-Allah, A. S. E. (2008). Effect of green alga cells extract as foliar spray on vegetative growth, yield and berries quality of superior grapevines. *American-Eurasian Journal of Agriculture & Environmental Science*, *4*(4), 427–433.

Abdel-Raouf, N., Al-Homaidan, A. A., & Ibraheem, I. B. M. (2012). Agricultural importance of algae. *African Journal of Biotechnology*, *11*(54), 11648–11658. doi:10.5897/AJB11.3983

Androutsopoulos, V. P., Hernandez, A. F., Liesivuori, J., & Tsatsakis, A. M. (2013). A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides. *Toxicology*, *307*, 89–94. doi:10.1016/j.tox.2012.09.011

Bhagavathy, S., Sumathi, P., & Jancy Sherene Bell, I. (2011). Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine*, *1*(1), S1–S7. doi:10.1016/S2221-1691(11)60111-1

Borowitzka, M. A. (1995). Microalgae as sources of pharmaceuticals and other biologically-active compounds. *Journal of Applied Phycology*, *7*(1), 3–15.

Bouchard, M. F., Bellinger, D. C., Wright, R. O., & Weisskopf, M. G. (2010). Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. *Pediatrics*, *125*(6), e1270–7. doi:10.1542/peds.2009-3058

Brennan, L., & Owende, P. (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, *14*(2), 557–577. doi:10.1016/j.rser.2009.10.009

Burkiewicz, K. (1987). Active substances in the media after algae cultivation. *Acta Physiologiae Plantarum*, *9*(4), 211–217.

Cannell, R. J. P., Owsianka, A. M., & Walker, J. M. (1988). Results of a large-scale screening programme to detect antibacterial activity from freshwater algae. *British Phycological Journal*, *23*(1), 41–44. doi:10.1080/00071618800650051

Catarina Guedes, A., Barbosa, C. R., Amaro, H. M., Pereira, C. I., & Xavier Malcata, F. (2011). Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. *International Journal of Food Science & Technology*, *46*(4), 862–870. doi:10.1111/j.1365-2621.2011.02567.x

Chojnacka, K., Saeid, A., Witkowska, Z., & Tuhy, Ł. (2012). Biologically active compounds in seaweed extracts - the prospects for the application. In *The Open Conference Proceedings Journal* (pp. 20–28).

CLSI. (2012). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard— Eleventh Edition. CLSI document M02-A11* (Vol. 32).

Cresswell, J. E., & Thompson, H. M. (2012). Comment on "A common pesticide decreases foraging success and survival in honey bees". *Science*, *337*(6101), 1453; author reply 1453. doi:10.1126/science.1224618

Debro, L. H., & Ward, H. B. (1979). Antibacterial activity of freshwater green algae. *Planta Medica*, *36*(4), 375–378.

Faheed, F. A., & ABD-EL Fattah, Z. (2008). Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of lettuce plant. *Journal of Agriculture & Social Sciences*, *4*(4), 165–69.

Fernández, V., & Eichert, T. (2009). Uptake of hydrophilic solutes through plant leaves: Current state of knowledge and perspectives of foliar fertilization. *Critical Reviews in Plant Sciences*, *28*(1-2), 36–68. doi:10.1080/07352680902743069

Fogg, G. E. (1983). The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Marina*, *26*, 3–14.

Foley, P. M., Beach, E. S., & Zimmerman, J. B. (2011). Algae as a source of renewable chemicals: opportunities and challenges. *Green Chemistry*, *13*(6), 1399–1405. doi:10.1039/c1gc00015b

Haynes, R. J., & Goh, K. M. (1977). Review on physiological pathways of foliar absorption. *Scientia Horticulturae*, *7*, 291–302.

Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Ruiz-López, M. A., Norrie, J., & Hernández-Carmona, G. (2013). Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *Journal of Applied Phycology*, *26*(1), 619–628. doi:10.1007/s10811-013-0078-4

Herrero, M., Cifuentes, A., & Ibanez, E. (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. *Food Chemistry*, *98*(1), 136–148. doi:10.1016/j.foodchem.2005.05.058

Ibtissam, C., Hassane, R., José, M., Seglar, D., Francisco, J., Vidal, G., … Mohamed, K. (2009). Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. *African Journal of Biotechnology*, *8*(7), 1258–1262.

Kannan, S., & Charnel, A. (1986). Foliar absorption and transport of inorganic nutrients. *Critical Reviews in Plant Sciences*, *4*(4), 341–375. doi:10.1080/07352688609382231

Kellam, S. J., & Walker, J. M. (1989). Antibacterial activity from marine microalgae in laboratory culture. *British Phycological Journal*, *24*(2), 191–194. doi:10.1080/00071618900650181

Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., … Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation*, *28*(4), 386–399. doi:10.1007/s00344-009-9103-x

Kumar, G., & Sahoo, D. (2011). Effect of seaweed liquid extract on growth and yield of *Triticum aestivum* var. Pusa Gold. *Journal of Applied Phycology*, *23*(2), 251–255. doi:10.1007/s10811-011-9660-9

Kumari, R., Kaur, I., & Bhatnagar, a. K. (2011). Effect of aqueous extract of *Sargassum johnstonii* Setchell & Gardner on growth, yield and quality of *Lycopersicon esculentum* Mill. *Journal of Applied Phycology*, *23*(3), 623–633. doi:10.1007/s10811-011-9651-x

Lee, J.-Y., Yoo, C., Jun, S.-Y., Ahn, C.-Y., & Oh, H.-M. (2010). Comparison of several methods for effective lipid extraction from microalgae. *Bioresource Technology*, *101 Suppl*(1), S75–7. doi:10.1016/j.biortech.2009.03.058

Mascarelli, A. (2013). Growing up with pesticides. *Science*, *341*(August), 740–741.

Mason, C. P., Edwards, K. R., Carlson, R. E., Pignatello, J., Gleason, F. K., & Wood, J. M. (1982). Isolation of chlorine-containing antibiotic from the freshwater cyanobacterium *Scytonema hofmanni*. *Science*, *215*(4531), 400–2. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6800032

Mata, T. M., Martins, A. a., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, *14*(1), 217–232. doi:10.1016/j.rser.2009.07.020

Metting, B. (1990). Microalgae applications in agriculture. *Developments in Industrial Microbiology*, *31*, 265–270.

Metting, B., & Pyne, J. W. (1986). Biologically active compounds from microalgae. *Enzyme and Microbial Technology*, *8*(7), 386–394. doi:10.1016/0141-0229(86)90144-4

Mostafalou, S., & Abdollahi, M. (2013). Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. *Toxicology and Applied Pharmacology*, *268*(2), 157–77. doi:10.1016/j.taap.2013.01.025

Nair, B. B., & Krishnika, A. (2011). Antibacterial activity of freshwater microalga (*Scenedesmus* sp.) against. *Journal of Biosciences Research*, *2*(4), 160–165.

Ordog, V., Stirk, W. A., Lenobel, R., Bancirova, M., Strnad, M., van Staden, J., … Nemeth, L. (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, *16*, 309–314.

Öztürk, Ş., Asl, B., & Beyatl, Y. (2006). Biological screening of microalgae isolated from different freshwaters of Turkey: Antimicrobial activity, viability and brine shrimp lethality. *Fresenius Environmental Bulletin*, *15*(10), 1232–1237.

Parrón, T., Requena, M., Hernández, A. F., & Alarcón, R. (2011). Association between environmental exposure to pesticides and neurodegenerative diseases. *Toxicology and Applied Pharmacology*, *256*(3), 379–85. doi:10.1016/j.taap.2011.05.006

Parsaeimehr, A., & Chen, Y. F. (2013). Algal bioactive diversities against pathogenic microbes. *Formatex*, (Mic), 796–803.

Plaza, M., Santoyo, S., Jaime, L., García-Blairsy Reina, G., Herrero, M., Señoráns, F. J., & Ibáñez, E. (2010). Screening for bioactive compounds from algae. *Journal of Pharmaceutical and Biomedical Analysis*, *51*(2), 450–5. doi:10.1016/j.jpba.2009.03.016

Prabakaran, P., & Ravindran, A. D. (2011). A study on effective lipid extraction methods from certain fresh water microalgae. *Elixir Bio Technology*, *39*, 4589–4591.

Pragya, N., Pandey, K. K., & Sahoo, P. K. (2013). A review on harvesting, oil extraction and biofuels production technologies from microalgae. *Renewable and Sustainable Energy Reviews*, *24*, 159–171. doi:10.1016/j.rser.2013.03.034

Pratt, R., Daniels, T. C., Eiler, J. J., Gunnison, J. B., Kumler, W. D., Oneto, J. F., … Strain, H. H. (1944). Chlorellin, an antibacterial substance from *Chlorella*. *Science*, *99*(2574), 351–352.

Pulz, O., & Gross, W. (2004). Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, *65*(6), 635–48. doi:10.1007/s00253-004-1647-x

Rodríguez-Meizoso, I., Jaime, L., Santoyo, S., Señoráns, F. J., Cifuentes, A., & Ibáñez, E. (2010). Subcritical water extraction and characterization of bioactive compounds from *Haematococcus pluvialis* microalga. *Journal of Pharmaceutical and Biomedical Analysis*, *51*(2), 456–63. doi:10.1016/j.jpba.2009.03.014

Santoyo, S., Rodríguez-Meizoso, I., Cifuentes, A., Jaime, L., García-Blairsy Reina, G., Señorans, F. J., & Ibáñez, E. (2009). Green processes based on the extraction with pressurized fluids to obtain potent antimicrobials from *Haematococcus pluvialis* microalgae. *LWT - Food Science and Technology*, *42*(7), 1213–1218. doi:10.1016/j.lwt.2009.01.012

Shaaban, M. M. (2001). Nutritional status and growth of maize plants as affected by green microalgae as soil additives. *OnLine Journal of Biological Sciences*, *6*(1), 475–479.

Shaaban, M. M., El-Saady, A.-K. M., & El-Sayed, A. E.-K. B. (2010). Green microalgae water extract and micronutrients foliar application as promoters to nutrient balance and growth of wheat plants. *Journal of American Science*, *6*(9), 631–636.

Shukla, A. C., & Gupta, A. B. (1967). Agriculture: Influence of algal growth-promoting substances on growth, yield and protein contents of rice plants. *Nature*, 744.

Stanier, R. Y., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order *Chroococcales*). *Bacteriological Reviews*, *35*(2), 171–205.

Tarakhovskaya, E. R., Maslov, Y. I., & Shishova, M. F. (2007). Phytohormones in algae. *Russian Journal of Plant Physiology*, *54*(2), 163–170. doi:10.1134/S1021443707020021

Verkleij, F. N. (1992). Seaweed extracts in agriculture and horticulture: A review. *Biological Agriculture and Horticulture*, *8*(4), 309–324.

Wijffels, R. H., & Barbosa, M. J. (2010). An outlook on microalgal biofuels. *Science*, *329*(5993), 796–9. doi:10.1126/science.1189003